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Establishing baseline mastitis parameters on commercial New Zealand sheep milking farms

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ABSTRACT

Like all dairy species, milk production in sheep depends on udder health, and mastitis (inflammation of the mammary glands, usually associated with infection), is one of the most important diseases of dairy animals. Mastitis negatively affects animal welfare, productivity and milk quality and is therefore an animal health priority for dairy sheep operations globally. Sheep milking has grown rapidly in New Zealand in the last 20 years. As a new industry, New Zealand lacks its own systematically collected data, forcing farmers and veterinarians to extrapolate from bovine research or studies conducted in overseas dairy sheep industries. Differences in breeds, environments, management systems and industry priorities mean that overseas findings may not be directly applicable to New Zealand.

In the 2022-2023 season, a research project was conducted to establish baseline parameters for udder health and milk quality in New Zealand dairy sheep. The objectives were to determine the incidence of clinical mastitis, the prevalence of subclinical mastitis and the bacterial causes of both; establish how clinical mastitis is treated and the outcomes that are achieved; characterise udder and teat conformation and health; describe ewe and bulk milk somatic cell counts and aerobic plate count; find somatic cell count thresholds for diagnosing intramammary infection (infection of the mammary tissue); explore the use of the rapid mastitis test and its correlation with somatic cell count; identify key risk factors for mastitis and elevated aerobic plate count; and determine associations between bulk milk somatic cell count and aerobic plate count.

The research was conducted on 20 commercial farms spread from North Waikato to Canterbury, purposively selected to represent a range of farm systems. These 20 farms comprised approximately half of the commercial dairy sheep farms in operation at the time. Farmer interviews were conducted to collect descriptive and risk factor data. Clinical mastitis

data and milk samples were collected by farm staff as cases were diagnosed according to a standardised definition (a change in the appearance of milk and/or signs of inflammation in the gland). Separately to this farmer work, the research team visited each farm at three points in the season, coinciding with early, mid and late lactation. Approximately 15 lactating ewes were randomly selected on each farm. Four udder morphology measures were scored on a 5-point scale: depth (the distance between the udder cleft and the abdominal wall), suspension (ratio of the width at the abdominal attachment to the height), separation (the extent of the udder cleft), and teat placement (determined from the vertical distance between the teat attachments and the most distal point of the udder). Teat length and width were measured. Teats and udders were assessed for presence of visible teat/udder inflammation, lesions and teat end hyperkeratosis, palpable consistency and lesions. The presence of a supernumerary teats and non-lactating glands ("half udders", subjectively judged to be producing a small volume of milk compared to the contralateral gland, or to be non-lactating) were recorded. Milk samples were collected to conduct the rapid mastitis test and measure somatic cell count, aerobic plate count and perform bacterial culture. Bulk milk somatic cell count and aerobic plate count data were collected from the 16 farms that sent milk to a processor. Subclinical mastitis was defined at the ewe level as a ewe having one or two bacteriologically positive glands and a SCC >500,000 cells/mL and/or a RMT score ≥1. Clinical mastitis data were collected for 236 cases and randomly selected ewe data were collected from a total of 893 ewes.

Farm practices varied widely, both between farms but also within farms across the season. Milk recording was only performed on 7/20 (35%) of study farms and ewe-level data were incomplete or absent on many farms. Wearing gloves during milking was mandatory on 40% of farms, and teat disinfection was used all season on 40% of farms.

The morphology of the ewe teats and udders was similar to that observed in overseas dairy sheep and varied substantially between farms, while pathology was rare, with all conditions having a prevalence <6%.

Clinical mastitis had a low incidence (2.3%) compared to New Zealand dairy cows, but consistent with overseas estimates for dairy ewes. While the incidence was low, clinical mastitis could be severe, with 25% of affected ewes having a fever and an overlapping 26% having depression, and only 15% of ewes recovering without lasting sequelae. Nearly half of all the clinical mastitis milk samples were culture negative. Including those in the denominator, *Streptococcus uberis* (14%), non-aureus staphylococci (12%), and *Staphylococcus aureus* (11%) were the most common isolates. *Streptococcus uberis* is a dominant cause of clinical mastitis in New Zealand dairy cows but it is not commonly reported in dairy ewes overseas.

Subclinical mastitis had a prevalence of 6.4%, which was substantially lower than the prevalence estimates reported in the EU, while recognising that variation in definitions and methods limits direct comparison. Milk samples collected from the randomly selected ewes had a geometric mean somatic cell count of 169,039 and a range of 2,000 to 34,953,000 cells/mL. The geometric mean somatic cell count was substantially higher than reports from European studies.

Bacteria were isolated from 5.5% of the glands of randomly selected ewes, with the most common species being non-aureus staphylococci (4.0% of glands) and *S. aureus* (0.6% of glands), consistent with the aetiology in the northern hemisphere. Moderate or severe teat end hyperkeratosis and udder asymmetry were confirmed as risk factors for subclinical mastitis. Although udder asymmetry is widely recognised as a clinical indicator of mastitis, this study

provides, to our knowledge, the first published evaluation of the association in dairy sheep using a defined udder assessment protocol.

A somatic cell count threshold of approximately 400,000 cells/mL had the greatest accuracy for diagnosing intramammary infection, but while it had a specificity of 0.88, its sensitivity was only 0.64. Mean $\log_{10}$ somatic cell count increased linearly with rapid mastitis test score but its agreement was only moderate (Kendall's tau = 0.47).

Elevated rapid mastitis test score and somatic cell count, positive milk culture and subclinical mastitis were identified risk factors for elevated ewe-level aerobic plate count. Bulk milk somatic cell count had a geometric mean of 659,491 cells/mL, which was in the mid-range of estimates from overseas studies, while bulk milk aerobic plate count exceeded 100,000 CFU/mL in 22.3% of consignments, peaking in August. High bulk milk aerobic plate count was not well predicted by somatic cell count. Clinical mastitis incidence, subclinical mastitis prevalence, and bulk milk quality targets were set based on the means of the best performing quarter of farms.

This thesis provides the first nationally representative mastitis and milk-quality baseline for New Zealand dairy sheep. It has confirmed that New Zealand dairy sheep mastitis is low-frequency but high-impact, with clinical cases concentrated around lambing with the potential for severe outcomes and poor prognoses. *Streptococcus uberis* is a relatively prominent cause of clinical mastitis among New Zealand dairy ewes compared to dairy ewes overseas. This shifts prevention efforts to early-lactation environmental control. Subclinical mastitis is caused by similar bacteria to overseas but the prevalence is low in New Zealand's relatively extensive, machine-milked systems, suggesting a structural advantage worth protecting as the industry intensifies. Mastitis should be considered when managing high bulk milk aerobic plate count, but the relationship between somatic cell count and aerobic plate count at the

bulk milk level is too weak for somatic cell count to serve as a management proxy. Currently, surveillance and decision-making are constrained more by data infrastructure than biology, making milk recording and repeat somatic cell count /rapid mastitis test protocols the most immediate area to develop on farm. Using this baseline information, future research can now be prioritised to address gaps, particularly in relation to causality, risk factors and diagnosis.

ABBREVIATIONS

Abbreviation	Definition
APC	Aerobic plate count
AUC	Area under the curve
BCS	Body condition score
BMSCC	Bulk milk somatic cell count
CFU/mL	Colony-forming units per millilitre
CI	Confidence interval
CMT	California Mastitis Test
DIM	Days in milk
ELPD	Expected log point-wise predictive density
ICC	Intraclass correlation coefficient
IMI	Intramammary infection
IQR	Interquartile range
LOO-CV	Leave-one-out cross-validation
MALDI-TOF	Matrix assisted laser desorption ionisation time-of-flight mass spectrometry
MPI	Ministry for Primary Industries
NAS	Non-aureus staphylococci
NPV	Negative predictive value
OR	Odds ratio
PCR	Polymerase chain reaction
PPV	Positive predictive value
RMT	Rapid mastitis test
ROC	Receiver operating characteristic
ROC AUC	Area under the receiver-operating characteristic curve
SCC	Somatic cell count
Se	Sensitivity
Sp	Specificity
SOP	Standard operating procedure
T	Trace (CMT or RMT scale)
TBC	Total bacterial count
YI	Youden's index

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1 INTRODUCTION

1.1 Background

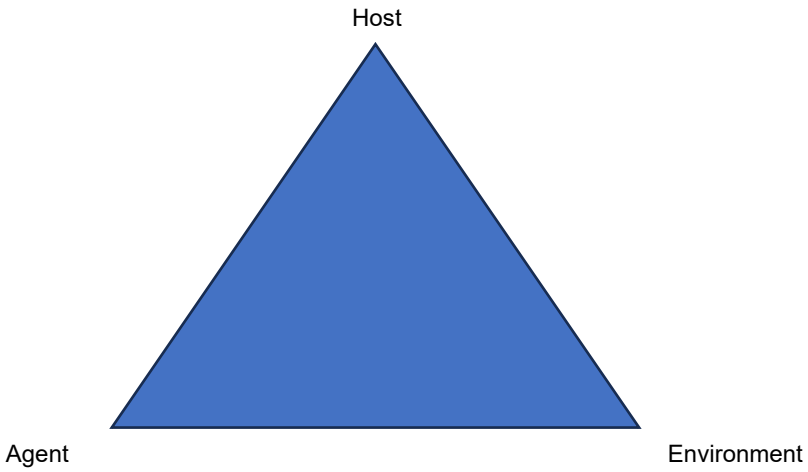
Sheep milking is a relatively new industry in New Zealand. Until the 2010s, there were fewer than 10 New Zealand sheep milking farms. The formation in 2014 of Maui Sheep Milk and Spring Sheep, two dairy companies that collect milk from their own farms as well as suppliers, saw a rapid growth in the number of farms. By 2019, there were 18 known operations nationally milking an estimated 22,000 ewes (Anonymous 2020a) and, between 2016 and 2020, the sector saw 12% annual growth in sheep numbers (Anonymous 2020b). Catalysed by access to a plant that can dry milk into powder at Innovation Park in Hamilton, Maui Sheep Milk and Spring Sheep had begun to collect, process and market sheep milk. At the same time, independent operators and small groups started or continued to operate, producing pasteurised milk, cheese, yoghurt, soap and other products.

As a new industry, there are knowledge gaps in many areas but also large opportunities to make significant improvements. Mastitis, meaning inflammation of the mammary gland, is the host's inflammatory response to damage of the mammary tissue. It is most often caused by bacterial infection (Gelasakis *et al*. 2015; Tassi *et al*. 2024; Ruiz-Romero *et al*. 2025), which is referred to as intramammary infection (IMI). Mastitis is known from overseas research to affect animal wellbeing and mortality, milk quantity, quality, and processing (Jaeggi *et al.* 2003; Leitner *et al.* 2004; Alba *et al.* 2019). However, we lack information on the impact and management of mastitis on New Zealand dairy sheep farms. The lack of local research leaves farmers and their advisors reliant on extrapolation from bovine mastitis knowledge or overseas dairy sheep research. This body of work provides the first nationally representative mastitis and milk quality baseline for mastitis in New Zealand dairy sheep.

1.2 Why New Zealand dairy sheep data are needed

As an infectious disease, mastitis fits the classical "epidemiological triangle" of host, agent and environment (Figure 1).

Figure 1: The epidemiological triangle



Each factor is likely to have distinctive features in New Zealand dairy sheep systems: host factors such as breed composition, udder/teat phenotype and lactation management; environmental factors including seasonal, pasture-based production, stocking rates and milking routines; and agent factors such as the local pathogen community and transmission ecology. These factors shape the risk of intramammary infection and its consequences for somatic cell count (the number of white blood cells per mL of milk; a routine milk quality measure), milk quality, and clinical and subclinical disease, and therefore require New Zealand data to develop effective control strategies. While there is a large international body of published dairy sheep mastitis research, its relevance to New Zealand is limited by differences in breeds, environments and farming systems.

Multiple specialist dairy breeds have been developed overseas over decades and even centuries, while the New Zealand flock has been assembled from dairy (e.g., East Friesian)

and non-dairy breeds (e.g., Poll Dorset and Coopworth), combined with varying proportions and types of overseas dairy genetics (e.g., Lacaune). New breeds have been developed for New Zealand conditions, including DairyMeade (Miles and Janet King), Southern Cross (Maui Sheep Milk) and Zealandia (Spring Sheep).

Farm systems can be described on a spectrum of intensive to extensive. The European Food Safety Authority defines dairy sheep farming systems as varying from "very extensive (such as pastorals with practices such as manual milking, seasonal breeding and one lactation/year) to very intensive (with machine milking, concentrate supplementation, year around breeding with three lactations in two years, etc.)" (European Food Safety Authority 2014). In this thesis, intensive and extensive are used as relative descriptors of management intensity rather than fixed categories. Grazing is common among New Zealand dairy sheep farms, with some being entirely pasture-based. Thus, New Zealand dairy sheep systems may be considered more intensive than local non-dairy sheep systems, owing to regular milking, closer day-to-day management, and more use of concentrated feeds (e.g., barley) to supplement pasture, but relatively more extensive than many overseas dairy sheep systems, which are more often characterised by housing, less grazing, and greater reliance on conserved forage and concentrate feeding. Although literature specifically pertaining to grazing ewes exists from Mediterranean regions such as Sardinia (Cuccuru *et al.* 2011), Italy (Bianchi *et al.* 2016), Spain (Gonzalo *et al.* 2002), and Greece (Vasileiou *et al.* 2019a), the industries in these countries are longer-established than in New Zealand, have different breeds, 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, largely grazing-based, and typically uses modern infrastructure (McCoard *et al.* 2023), and machine milking is standard. These differences in farm system, management, and industry stage mean that findings from dairy sheep overseas are not always transferable to New Zealand conditions.

Most New Zealand dairy cow operations are pasture-based. Therefore, with an estimated 4.67 million cows on 10,601 farms in the 2022-2023 season (DairyNZ and Livestock Improvement Corporation 2023), the New Zealand bovine dairy industry provides a useful local reference point when considering mastitis in the emerging New Zealand dairy sheep industry. Mastitis is an important disease of New Zealand dairy cows, as seen by an estimated incidence of 10.0% among cows calving between 8 July and 21 August 1997 (McDougall 1998) and 14.8% for the entire lactation (Petrovski *et al*. 2009). It is well-established that *Streptococcus uberis* is a major cause of clinical mastitis, while *Escherichia coli* is a rare cause of clinical mastitis for New Zealand dairy cows (McDougall 2002). Although *Strep. uberis* is also an important cause of clinical mastitis in England and Wales, the dominant pathogens reported elsewhere vary and may include *E. coli* (which is also an important cause in England and Wales), *Staphylococcus aureus*, *Strep. dysgalactiae*, *Mycoplasma*, and *Klebsiella* (Zadoks and Fitzpatrick 2009). The aetiology in New Zealand dairy cows therefore appears to differ from that reported in some other countries, likely reflecting, at least in part, differences in production systems and management. The pasture-based seasonal calving system concentrates many cows into a relatively short calving and early-lactation period, during which they are managed in groups at pasture, often under wet winter and spring conditions that may favour exposure to environmental mastitis pathogens such as *Strep. uberis*. Furthermore, the seasonal cessation of milking in the winter may interrupt the spread of contagious pathogens, while antibiotic dry cow therapy may further reduce the prevalence of existing intramammary infection, thus reducing their relative importance as causes of mastitis. Consistent with the significance of environmental exposure in the New Zealand system, Lopez-Benavides *et al*. (2007) identified environmental niches of *Strep. uberis* on a New Zealand pasture-based dairy farm. They reported higher concentrations of *Strep. uberis* on farm tracks that had higher cow traffic than tracks with lower traffic, and that *Strep. uberis*

was only detected in paddocks after cow grazing. They also found a relationship between farm track and milk isolates. These findings suggest that New Zealand cows are repeatedly exposed from the farm environment rather than mainly through contagious spread. Accordingly, given that dairy sheep are also largely pasture-based and walk from the paddock to the parlour for milking, findings from overseas mastitis research should not be assumed to apply directly to New Zealand dairy production systems.

It should also not be assumed that our understanding of mastitis in dairy cows or goats, or non-dairy sheep, can be applied to dairy sheep, even in New Zealand systems. While dairy cows may be managed under similar, predominantly pasture-based systems to dairy sheep in New Zealand, dairy goats are not: they are predominantly farmed intensively, being housed in barns with wood chip- or sawdust-lined floors and their food brought to them (Scholtens *et al*. 2017). Yet under the same management conditions, there are important between-species differences. In a review of mastitis of dairy small ruminants, Bergonier *et al*. (2003) argued that small ruminant mastitis has important differences to that in cows. They identified staphylococci as the principal intramammary pathogens, with *Staphylococcus aureus* more common in clinical cases and coagulase-negative staphylococci more common in subclinical infections. They further described small-ruminant mastitis as typically chronic and contagious, with mammary and cutaneous carriage acting as important reservoirs and transmission occurring mainly during milking. Clinical mastitis was reported to have an annual incidence usually below 5%, while somatic cell count (SCC) was considered a more reliable indicator of subclinical mastitis in sheep than in goats. In addition to differences in mastitis epidemiology, dairy sheep differ from cows and goats in basic milk characteristics. Sheep milk is generally more concentrated, with higher total solids, fat, protein, and mineral contents than cow or goat milk (Hadjipanayiotou 1995). Small-ruminant milk contains cytoplasmic particles that complicate SCC interpretation, especially in goats, although the

traditional apocrine/merocrine classification is not described consistently for sheep (Kaskous *et al*. 2022). These interspecies differences in milk composition and mammary physiology further caution against direct extrapolation of udder-health findings between species.

New Zealand non-dairy sheep are unlikely to be a useful reference for New Zealand dairy sheep. Dairy sheep are managed more intensively, have their milk harvested and the production objectives are different. Compared to non-dairy sheep, dairy sheep are brought in for milking, handled more often, grouped differently, and exposed to milking equipment and routines that can influence teat condition and transmission risk. Given that Bergonier *et al*. (2003) described mastitis in small ruminants as typically chronic and contagious, the act of milking means that the incidence of new infections and the bacterial causes may differ. Furthermore, it is unclear if earlier removal of lambs from dairy ewes changes the epidemiology of mastitis. For non-dairy sheep, the primary role of the udder is to rear lambs while, in dairy sheep, milk is also harvested for human consumption. That means dairy flocks usually have stronger selection pressure on udder conformation, milkability, milk yield, and milk quality, and much closer ongoing monitoring of udder health, such as SCC testing of ewes and bulk milk.

Because we lack clear information on the true magnitude of mastitis, diagnostic test thresholds, bacterial cause, risk factors and targets, New Zealand farmers and advisors cannot easily judge whether mastitis is significant on their farms, prioritise intervention practices or easily use diagnostic tools. They may be missing opportunities to manage mastitis more effectively, such as by targeting New Zealand-specific risk factors. This programme fills that gap by systematically collecting data directly from New Zealand farms to establish baseline parameters, clarify the use of diagnostic tests and setting targets and practical recommendations for farmers and the wider industry. These data will also provide a novel

international contrast and broaden a literature otherwise centred on the northern hemisphere with its more intensive production models.

1.3 Mastitis in dairy sheep

Mastitis is either clinical (there are observable signs such as a change in the milk's appearance or swelling and pain of the affected gland) or subclinical (only detectable by testing but can cause production losses and milk quality problems). Clinical mastitis is generally an acute condition, so it is normally measured in terms of incidence proportion ("incidence"), which is the number of new cases in a given time period divided by the number of animals in the population. Prior to the research presented here, the incidence in New Zealand dairy flocks was unclear. French researchers noted in a review that the incidence within a dairy sheep lactation or year was typically less than 5% and comprised largely of sporadic cases, though larger-scale outbreaks had been reported (Bergonier and Berthelot 2003). In 1968, a New Zealand cross-sectional survey of non-dairy ewes, in which 19,427 ewes were manually examined in either August-September or January-February, reported signs of clinical mastitis and udder defects in 1.7% of the ewes (Quinlivan 1968). While these figures are low compared to New Zealand dairy cows (McDougall 1998; Petrovski *et al.* 2009), clinical mastitis in dairy sheep can be severe. Veterinary examinations of mastitic ewes in a Norwegian study of dairy sheep found moderate or severe systemic signs in half of the 509 ewes detected with clinical mastitis, gangrene in 9% of glands, and pyrexia in more than half of affected ewes (Mork *et al.* 2007). Treatment information for ovine clinical mastitis is sparse and there are no intramammary products registered for lactational or dry ewe therapy in New Zealand. Several injectable antimicrobials are registered for sheep, with only a few stating a milk withholding period, so, in many instances, long milk withholding periods of 35 days (the default withholding period for the off-label use of medicines in New Zealand) are applied, limiting the economic value of treatment due to

the prolonged milk discard period. New Zealand research is needed to understand the importance of clinical mastitis and how to optimally manage, treat, and prevent it.

Subclinical mastitis is often a chronic condition, so it is typically measured in terms of prevalence (the proportion of animals infected at a given time point). On one hand, it is more challenging to identify than clinical mastitis without diagnostic tools because it is not obvious to the naked eye. On the other hand, routine milk quality monitoring can bring subclinical mastitis to farmers' attention. Milk somatic cell count (SCC; the number of white blood cells per ml of milk), the rapid mastitis test (RMT, a ewe-side test that semi-quantitatively estimates SCC) and bacterial culture are often used to monitor milk quality and diagnose and manage mastitis. The SCC increases in response to intramammary infection (Albenzio *et al.* 2019). Research definitions of subclinical mastitis vary between studies but require some combination of a positive milk culture (i.e., a microorganism was grown), usually in the presence of elevated SCC (Fthenakis 1994; Lysitsas *et al.* 2024), RMT (Las Heras *et al.* 1999), or elevated milk neutrophil and lymphocyte (types of white blood cell) proportions (Vasileiou *et al.* 2018). A nationwide survey of 111 Greek sheep milking farms found that 26% of ewes had subclinical mastitis (Vasileiou *et al.* 2018). Earlier work in Greece found subclinical mastitis prevalences of 5%, 11%, and 17% in early, mid, and late lactation, respectively (Fthenakis 1994). Las Heras et al. (1999) estimated the prevalence to be 34% among Spanish ewes. Despite affected ewes appearing healthy, subclinical mastitis has been clearly shown to negatively impact productivity and milk quality. Israeli researchers studied 36 ewes with one healthy gland and one gland affected by subclinical mastitis and showed that production was halved in the affected gland (Leitner *et al.* 2004). Brazilian researchers found a similar reduction in milk quantity, as well as a decline in quality, with large increases in SCC, among 30 RMT-positive Lacaune sheep (Alba *et al.* 2019). To our knowledge, there were no robust, published New Zealand studies on subclinical mastitis, how well SCC can be

utilised to indicate infection and how feasible the use of RMT may be to aid in diagnosis prior to this project, leaving farmers and their advisors without a framework to follow.

Understanding the aetiology (the infectious causes) is essential for controlling mastitis. Anecdotally, many New Zealand dairy sheep farmers have conducted individual investigations into mastitis for their own farms, but, to our knowledge, the aetiology had not been systematically studied in a coordinated fashion using consistent methodology. Staphylococci are believed to be the most common mastitis pathogen of dairy sheep overseas (Fthenakis 1994; Vasileiou *et al.* 2018; Michael *et al.* 2023). With our unique management systems, genetics, and climate in New Zealand, the causes must be established locally. Understanding the causes will help select the best intervention practices. This should be done in a prospective, structured manner because relying on spontaneously collected milk samples is prone to bias (since farmers are more likely to sample certain cases).

Once mastitis has been quantified and characterised, an understanding of its risk factors is necessary to control it. Returning to the epidemiological triangle, mastitis is a multifactorial disease that is often associated with environmental factors such as milking machinery, teat end health, milking procedures and udder hygiene (Vasileiou *et al.* 2019b), as well as host factors such as udder conformation (Huntley *et al.* 2012; Marshall *et al.* 2023). The value of management factors as simple as teat spraying and wearing gloves during milking has never been systematically appraised for dairy sheep in New Zealand.

Somatic cell counts are a key tool for managing mastitis and require baseline data for New Zealand. The SCC is often used for two purposes in dairy operations: the diagnosis of mastitis in individual animals by way of milk recording, and the monitoring of mastitis and milk quality at the farm level via routine measurement of bulk milk SCC. The latter is typically performed by processors who purchase bulk milk from suppliers. Anecdotally, there

is a perception in New Zealand that SCC is high in ewes compared to cows, but this has not been clarified because there are no published studies describing SCC at scale at either the ewe or bulk milk level in New Zealand. Understanding the central tendency, range, and between-farm variation in ewe and bulk milk SCC is necessary to define what is normal and set targets based on the best performing farms.

When using SCC to diagnose mastitis in individual ewes, it is practical to apply a threshold to separate ewes into high-SCC (and thus deemed more likely to have mastitis) and low-SCC (deemed less likely to have mastitis) groups. Currently, it is unclear what threshold to apply for New Zealand ewes. Thresholds have been proposed overseas (Berthelot *et al.* 2006; Riggio *et al.* 2013; Knuth *et al.* 2019), but these vary widely based on the prevalence and aetiology of each study population and according to the intended use of the threshold. A lower threshold favours sensitivity (fewer infected ewes are missed and thus erroneously diagnosed as uninfected), but it also increases the likelihood that uninfected ewes are classified as positive. Conversely, a higher threshold favours specificity (fewer uninfected ewes are erroneously diagnosed as infected), but it increases the likelihood that infected ewes are missed. Thus, local work is needed to determine thresholds relevant to New Zealand conditions that minimise diagnostic errors. False negatives (not detecting true mastitis cases) mean that mastitic ewes remain in the flock, posing a contagion risk to uninfected ewes. False positives (diagnosing uninfected ewes as having mastitis) risks the unnecessary treatment or culling of healthy ewes.

The RMT is used anecdotally on many New Zealand dairy sheep farms, and its correlation with SCC has been validated for sheep in a small New Zealand study (McDougall *et al.* 2001). In dairy cattle, the RMT is widely used as a rapid indicator of elevated SCC, although its agreement with SCC and intramammary infection is imperfect (Abdulkhader *et al*. 2022). Further validation is therefore needed to give farmers confidence in using the RMT as a ewe-

side tool for detecting high-SCC ewes. Greater confidence will drive use and therefore contribute to detecting mastitis and reducing the burden of disease.

Teat and udder conformation are critical for managing mastitis. In the progression from start-up flocks consisting of mixed breeds to consolidated flocks specialised for dairy farming, farmers often focus on teat and udder conformation because these traits impact milkability (how efficiently and easily milk can be harvested from the ewe), and consequently productivity, but also teat and udder health, and milk quality. Strong gland separation, well-attached udders, and higher udder depth scores (less pendulous udders) have been associated with lower SCC (Huntley *et al.* 2012; Marshall *et al.* 2023). These morphology traits are heritable, making selection of replacement ewes an important tool for farmers. Fernández *et al*. (1997) studied Churra breed ewes and determined heritabilities of 0.16, 0.17, 0.24, 0.18 and 0.24 for udder depth, udder attachment, teat placement, teat size, and udder shape, respectively. Teat and udder morphology have not been systematically described for New Zealand ewes across a large number of farms. Robust morphology data are needed to inform efforts to improve udder conformation by identifying the areas with the greatest potential for improvement (when combined with heritability) and the areas with the greatest variance. This baseline information can also be used to gauge improvements across time and develop a practical and rapid scoring system optimised for New Zealand conditions.

Other health conditions of the teats and udders may affect mastitis, such as wounds, inflammation of the skin, and skin infections. In particular, teat end hyperkeratosis, an excessive thickening of the teat end skin caused by the process of being machine milked under suboptimal conditions, is a known mastitis risk factor (Vouraki *et al.* 2018). While performing visual assessments of teat and udder morphology, data on visual and palpable pathology can be collected to describe the health of New Zealand dairy ewes' teats and udders and determine if there were any consistent pathologies that warrant attention at the

industry level. Individual farmers may manage teat and udder conditions separately, but a coordinated research program is needed to robustly understand the prevalence of pathology across the industry and share the information.

Bulk milk data are valuable for monitoring and managing mastitis and assuring milk quality. Bulk milk SCC is a widely used indicator of udder health and milk quality, and it has been shown to correspond to the prevalence of subclinical mastitis in dairy sheep (Fthenakis 2023). Aerobic plate count (APC) assesses the bacterial load of the milk, which is typically associated with milking hygiene, plant sanitation and refrigeration effectiveness (Jayarao *et al.* 2004). In New Zealand, all dairy farmers producing milk for human consumption are required by the Ministry for Primary Industries (MPI) to perform routine bulk milk testing of a suite of parameters, including SCC and APC, regardless of whether the farmer supplies a processor. The MPI action limits for SCC and APC of raw bulk sheep milk are 1,500,000 cells/mL and 100,000 CFU/mL, respectively (Anonymous 2025). These action limits are regulatory trigger points: results exceeding them require notification and corrective action procedures under the farm dairy's Risk Management Programme, rather than defining biological thresholds for mastitis or milk hygiene. Little is known about typical SCC and APC profiles in the bulk milk of New Zealand farms, how much they vary across time and between farms, and whether they are different to those of overseas flocks. This knowledge gap limits benchmarking of farm performance and impedes the establishment of clear quality targets, which are needed to signal when to act at a farm level.

Secondary to mastitis, there is no known published peer-reviewed work describing the attributes of New Zealand dairy sheep farms, such as flock size, staff numbers, animal management, productivity and milking infrastructure and management. The research project aimed to accumulate a large amount of general farm information to explore risk factors. This information, collected by in-person interview, would also make a valuable contribution to the

literature on dairy sheep farming in New Zealand in its own right and serve as useful reference for the journey a country undertakes when establishing a dairy sheep industry.

1.4 Aerobic plate count

Anecdotally, high bulk milk APC (typically defined in the New Zealand dairy sheep industry as counts exceeding 100,000 CFU/mL) have occurred frequently and been a challenge to resolve on several New Zealand dairy sheep farms. Producers report that high counts can 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 RMT and removal of high-RMT ewes from supply. This has led to the hypothesis that mastitis may be a contributor to high bulk milk APC on dairy sheep farms, as has been shown for dairy cattle (Zadoks *et al*. 2004). However, this hypothesis has not been formally tested for New Zealand dairy sheep, and it is possible that changes in APC were coincidental. Describing ewe-level APC and identifying its risk factors may help to identify whether individual ewes can be the principal source of high bulk milk APC in pastoral systems, thus helping farmers address the problem. There is no known published international research on the relationship between APC and mastitis at the ewe level.

At the bulk milk level, a positive correlation between SCC and total bacterial count (TBC) has been demonstrated (de Garnica *et al.* 2013; Lianou *et al.* 2021). If bulk milk SCC is positively associated with bulk milk APC, control of SCC may contribute to managing APC, since they may have a causal relationship or share common risk factors, providing a useful tool to farmers. It is therefore prudent to describe bulk milk APC and determine if it is associated with bulk milk SCC so that farmers and the wider industry can develop an evidence-based method for managing high bulk milk APC.

1.5 Objectives

At a high level, the aim of this research project was to provide the New Zealand sheep industry with baseline mastitis and APC data that are specific to our farming systems. The first step is to describe the status quo. This will enable farmers, advisors and stakeholders to take an evidence-based approach to managing mastitis and milk quality for the benefit and sustainability of the industry. The research was therefore optimised for external validity to generate data that represent a wide range of farming systems in New Zealand. The conclusions are therefore more generalisable than data collected from a single farm or farms that are more accessible or better set up for research.

With input from farmers, processors and experts, an industry-funded mastitis research project was developed and approved as a PhD programme. Massey University was the first choice of university because of its reputation for high quality veterinary research, and the project aligned with Massey's research themes of Future Food Systems (food production and quality) and Resource Development and Management (mastitis has been shown to substantially inflate the environmental footprint of a litre of milk).

The specific objectives were to:

1. Perform a descriptive analysis of New Zealand sheep milking farms based on farmer interviews.
2. Characterise udder and teat conformation and health.
3. Determine the incidence and describe the aetiology of clinical mastitis and describe how farmers treat clinical mastitis and the outcomes.
4. Determine the prevalence, describe the aetiology and identify risk factors for subclinical mastitis.

5. Describe ewe-level somatic cell count and aerobic plate count and their associations with intramammary infection, determine the correlation between the rapid mastitis test and somatic cell count, find somatic cell count thresholds for diagnosing intramammary infection, and identify risk factors elevated ewe-level aerobic plate count.
6. Describe the distributions of bulk tank somatic cell count, aerobic plate count, and determine their association.
7. Generate a review of the research and recommend benchmarks, practices and research priorities for the future.

Objective 1 establishes how New Zealand dairy sheep farms operate, providing context for the remaining objectives and identifying themes that inform research strengths, limitations, and future opportunities. Internationally, systematic farm-level descriptions are sparse, and none exist for New Zealand based on standardised data collection, meaning research may not align with what is practically needed and achievable on farm. Furthermore, many aspects of mastitis and milk quality are downstream of general farm factors. Objective 2 yields information on important factors that influence farm milk harvesting efficiency and udder health, which are likely to be causally linked to mastitis and subsequently to bulk milk aerobic plate count. Work of this kind has not previously been done at scale either internationally or locally for dairy sheep. Not having this basic information makes it difficult to place teat and udder conformation in perspective as mastitis risk factors and challenging to improve them without a starting point. Objective 3 provides the first clear information on the size of the problem of clinical mastitis in New Zealand and lays groundwork on how to tackle it. There are no multi-farm clinical mastitis studies in dairy sheep reflecting a pastoral, seasonal production system of comparable scale to our knowledge. Objectives 4 and 5

similarly quantify the currently unknown prevalence of subclinical mastitis and therefore its importance in New Zealand, as well as providing necessary information regarding diagnostic tools and guidelines for farmers and advisors. These objectives also initiate the first systematic exploration of drivers of high bulk milk aerobic plate counts in New Zealand (including the first known exploration at the ewe level internationally), a priority issue raised by farmers. Using overseas data may lead to incorrect ranking of control priorities or inappropriate diagnostic test thresholds. Objective 6 produces the first systematic descriptions of bulk milk SCC and APC for New Zealand farms, allows targets to be set based on the best-performing farms under relevant New Zealand conditions, and helps farmers know what information is useful when managing high bulk milk aerobic plate count. Objective 7 makes the first, evidence-based recommendations for managing mastitis and future work for the New Zealand dairy sheep industry. This thesis has an emphasis on establishing baselines. Therefore, hypothesis-testing components within Objectives 4–6 (risk factors and associations) should be interpreted as exploratory. Collectively, Objectives 1–6 trace the pathway from system-level management and ewe phenotype through udder infection outcomes to bulk milk quality, enabling Objective 7's prioritisation.

Together, these objectives provide the first comprehensive baseline on mastitis and milk quality in New Zealand dairy sheep, contribute a novel pastoral-system perspective to the international literature, and establish a clear platform for targeted intervention and longitudinal research. Evidence-based targets can be set, initial diagnostic thresholds (SCC) are available, interventions can be ranked, and important mastitis management practices and research priorities can be recommended. While the research is primarily motivated by helping New Zealand farmers, it also gives insights into an industry during its establishment phase, which may serve other countries that go through the same journey. The novel learnings are

useful to other pastoral industries but also serve as a comparative model to more conventional intensive industries.

1.6 Thesis structure

Excluding the introductory chapter (Chapter 1), this thesis has three phases. Phase 1 (Chapters 2-7, objectives 1-6) follows a progression from describing NZ dairy sheep farms and ewe udder phenotype, to quantifying clinical and subclinical mastitis, evaluating diagnostic indicators, to exploring bulk milk quality. It contains mostly baseline descriptive information, but Chapters 5, 6, and 7 also test hypotheses about subclinical mastitis risk factors (Chapter 5), elevated aerobic plate count risk factors (Chapter 6) and the relationship between bulk milk somatic cell count and aerobic plate count (Chapter 7). Phase 2 (Chapter 8, objective 7), converts these findings into industry benchmarks and priorities. Phase 3 (Chapter 9) synthesises the findings from phases 1 and 2. The thesis ends with an appendix containing information on how farms were identified and recruited and relevant parts of the protocol on farmer and technician training, and collection, handling and processing of milk samples up to the point where the samples were sent for testing.

The links between knowledge gaps, study objectives and thesis chapters are summarised in Table 1.

Table 1: Links between knowledge gaps, study objectives and thesis chapters.

Knowledge gap	Research objective	Thesis chapter
Background knowledge		
General farm descriptive information	1	2
Teat and udder conformation and pathology	2	3
Mastitis burden and diagnostics		
Clinical mastitis incidence	3	4
Subclinical mastitis prevalence	4	5
Bacterial causes of mastitis	3, 4	4, 5
Mastitis risk factors	4	5
Somatic cell count distributions	5	5, 6
Rapid mastitis test validity	5	6
Ewe-level somatic cell count thresholds	5	6
Association between mastitis and aerobic plate count	5	6
Bulk milk quality and benchmarks		
Bulk milk somatic cell count and aerobic plate count distributions	6	7
Association between bulk milk somatic cell count and aerobic plate count	6	7
Recommended practices and research		
Recommended practices	7	8
Research priorities	7	8

Chapters 2-8 have either been published, been accepted and are in press, or have been submitted to a scientific journal. The status of each chapter is summarised in Table 2 below. The chapters that have been published or submitted to a scientific journal have been expanded on slightly in this thesis, the tables and figures have been moved so they are embedded in the body of the article, and the references and fonts have been formatted consistently. The spelling conventions of each journal have been preserved. Because each article was submitted or published independently, there is some repetition, particularly in the methods and discussion sections.

Table 2: List of thesis chapters and their publication details at the time of thesis submission (December 2025).

Chapter	Publication	Journal	Status
1. Introduction	Not written for publication		
2. Profile of New Zealand dairy sheep farms	Chambers G, Laven R. A profile of New Zealand dairy sheep farms in the 2022–2023 season. Forthcoming.	New Zealand Journal of Agricultural Research	In review
3. Teat and udder conformation and health	Chambers G, Lawrence KE, Ridler AL, Laven RA. Teat and udder morphology and pathology of New Zealand dairy ewes. 2025.	New Zealand Veterinary Journal	Published
4. Clinical mastitis - incidence, aetiology, treatment and outcomes	Chambers G, Laven R, Grinberg A, Ridler A, Velathanthiri N. An observational study of farmer-reported clinical mastitis in New Zealand dairy ewes. 2024.	New Zealand Veterinary Journal	Published
5. Subclinical mastitis - prevalence, aetiology and risk factors	Chambers G, Lawrence K, Grinberg A, Velathanthiri N, Ridler A, Laven R. Subclinical mastitis in New Zealand grazing dairy ewes 1: Prevalence and risk factors. 2026.	Journal of Dairy Science	In press
6. Subclinical mastitis - somatic cell count, rapid mastitis test and aerobic plate count	Chambers G, Lawrence K, Grinberg A, Velathanthiri N, Ridler A, Laven R. 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. 2026.	Journal of Dairy Science	In press
7. Bulk milk somatic cell count and aerobic plate count	Chambers G, Lawrence K, Laven R. Bulk milk somatic cell count and aerobic plate count on New Zealand dairy sheep farms. Forthcoming.	New Zealand Journal of Agricultural Research	In press
8. Narrative review and recommended benchmarks, practices and research priorities	Mastitis control in New Zealand dairy sheep: recommended benchmarks, practices and research priorities from a baseline national research programme. Forthcoming	New Zealand Veterinary Journal	In review
9. Synthesis	Not written for publication		

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2 A PROFILE OF NEW ZEALAND DAIRY SHEEP FARMS IN THE 2022–2023 SEASON

To help farmers manage mastitis, it is important to understand how farms operate so that research is focused on areas that can be practically addressed on farm. General farm information is also useful for generating hypotheses, particularly about risk factors for mastitis. A large amount of general descriptive farm data was collected in the course of this study. It was summarised in an article that was submitted to the New Zealand Journal of Agricultural Research on 17 October 2025, to serve as a reference point for industry stakeholders.

This article was submitted after all the other articles except the narrative review, and it was in peer review at the time this thesis was written. However, it is presented first to set the scene for the subsequent chapters and give the reader an impression of the size and management conditions of New Zealand dairy sheep farms, and their variability.

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A profile of New Zealand dairy sheep farms in the 2022–2023 season

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2.1 Abstract

New Zealand's sheep-milking sector is young. We conducted a cross-sectional study of 20 commercial farms (~50% of the industry) in 2022–2023. Using a standardised questionnaire, in-person interviews (May–June 2023) with owners/managers captured information on farm scale, staffing, lambing and lamb management, milk harvesting, milking facilities/procedures, and animal health practices. Farms had operated a median of four seasons (calendar years). Practices varied widely, particularly in lamb removal timing and milk-harvesting period; most farmers who supplied milk to processors separated lambs at ≤10 days of age, while on-farm processors separated lambs and ewes later and ran more flexible seasons. Staffing did not scale consistently with flock size; automation was partial (in-shed feeding was common, automatic cup removers were present on one-third of farms, and no farms had automatic teat sprayers). Common features were spring lambing, predominantly outdoor management, selenium supplementation, and clostridial vaccination. Milk recording and ewe data were limited. We present a New Zealand-specific baseline and highlight opportunities: strengthen ewe and milk data; evaluate staffing and automation; clarify effectiveness and practicality of milking hygiene interventions; and implement evidence-based trace element and leptospirosis risk management. Addressing these will support benchmarking, genetic gains, efficiency and animal health under New Zealand's pasture-based systems.

2.2 Introduction

Sheep milking is a relatively new industry that has grown significantly over the last 10 years in New Zealand. In 2019, there were 18 known commercial farms milking an estimated 22,000 ewes (Anonymous 2020). Between 2016 and 2020, there was an estimated 12% annual growth in dairy sheep numbers (Anonymous 2020). A small number of farms have milked sheep at times for some decades, but the establishment between 2010 and 2020 of companies that process the milk of supplier farms accelerated the growth (McCoard *et al.* 2023b).

There is a long history of sheep milking internationally, particularly in Asia, Europe and Africa (Pulina *et al*. 2018). Although this global context is important, much of the published literature most relevant to intensive and semi-intensive dairy sheep production originates from Mediterranean and European systems, where several specialised dairy breeds have been developed (Haenlein 2007). A wide range of farming systems exist, from extensive to intensive (Carta *et al.* 2009). In France, Greece, Italy and Spain, which produce 46% of the milk in the Mediterranean and Black Sea regions, the industry is generally semi-intensive to intensive (Pulina *et al.* 2018), and much of the published literature on dairy sheep originates in these countries. In contrast, New Zealand dairy sheep farms are predominantly spring-lambing and pasture-based, with sheep managed largely or entirely outdoors. Overseas research may not be generalisable to New Zealand conditions, so we need New Zealand-specific research, especially in relation to health and welfare.

In the 2022-2023 season, 20 commercial New Zealand dairy sheep farms were enrolled in a mastitis research programme (Chambers *et al.* 2024, 2025a, 2025b, 2025c), representing approximately 50% of the commercial farms operating at the time. Data were collected on mastitis, milk quality (somatic cell count, rapid mastitis test, aerobic plate count, bacterial

culture), teat and udder conformation and disease, bulk milk quality, and risk factors for subclinical mastitis and elevated ewe aerobic plate count. Interviews with farm owners or managers were conducted to gather management and herd-level information. In the process of collecting these data, substantial general farm information was also collected, providing a valuable, systematically collected snapshot of the industry at that time.

The purpose of this article is to present the general farm information that was collected during the 2022-2023 season. Our objectives are to describe the scale of the farms, how many seasons the farms had been in operation, staff numbers, seasonality, lambing management, milk production, milk harvesting and animal health. This information can be used to make evidence-based decisions at the industry level, draw comparisons between farms, and serve as a benchmark to compare against future research.

2.3 Materials and methods

This study was part of a wider project focusing on clinical mastitis on commercial New Zealand dairy sheep farms. It was identified as low risk by the Massey University Human Ethics Committee (number 4000026321).

2.3.1 Study farms

We aimed to recruit a mixture of farms (including independent producers and those that supplied dairy sheep processors) across different regions in New Zealand. Farms were thus convenience selected based on these criteria and the willingness of the farmers to participate and comply with the study procedures, provide access to flock and sheep level demographic data, and share mastitis records. Farmers affiliated with the three milk processing companies that collected milk and processed it off-farm were contacted via the processors, and independent operators were contacted directly. To satisfy the sample size requirements of other aspects of the mastitis research project, 20 farms were recruited (approximately half of

the estimated 40-50 commercial dairy sheep farms operating in New Zealand in the 2022-2023 season). This was based on calculating the prevalence of subclinical mastitis with 95% confidence that the estimate was within 6.8% (absolute) of the true population value (Chambers *et al.* 2025b).

The 20 study farms were located in the Waikato (n=12) and Wairarapa (n=2) regions of the North Island, and the Canterbury region (n=6) of the South Island (Figure 1). All of the milk from 16 farms was sent to a commercial processor (n=3 processors, two in the North Island and one in the South Island) off farm, while three farms processed their own milk and one farm both processed its own milk and supplied milk to a processor.

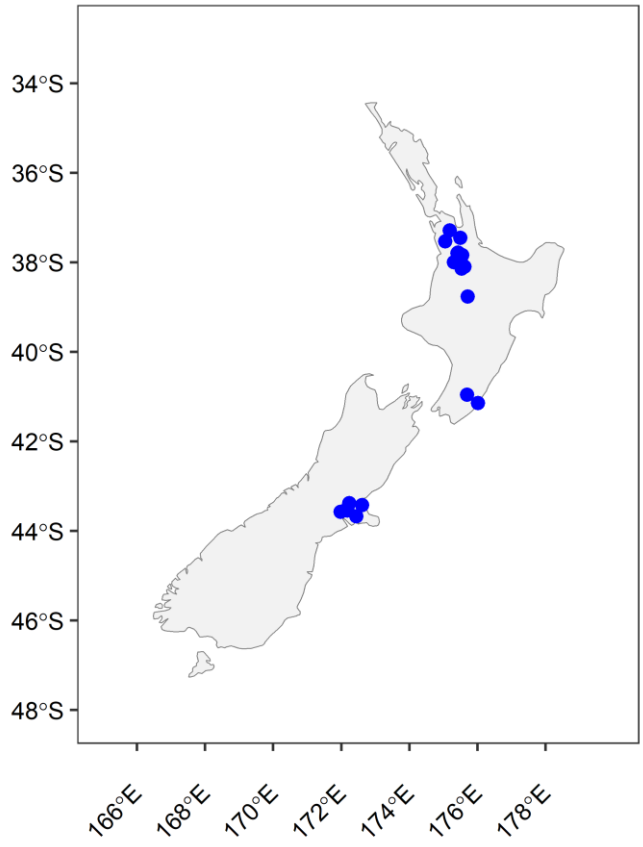

Figure 1: Locations of 20 farms in a study of New Zealand dairy sheep farms in the 2022-2023 season.

2.3.2 Data collection and statistical analysis

All data were collected using a standardised questionnaire. Each farm owner or manager was visited for a two- to three-hour interview at the farm, conducted in person by a single interviewer (GC), between 08 May and 13 June 2023. The questions were grouped into four categories: general farm information, milk production for the previous (2021-2022) season (the final 2022-2023 season production statistics were not available for all farms at the time of the interview), milk harvesting, and flock selenium management and vaccination practices (Table 1). Open and closed responses were entered into a spreadsheet (Excel, Microsoft Corporation, Redmond, WA) and all data were analysed in RStudio using R (v4.2.2) (R Core Team 2023). When a range was given by the interviewee, the midpoint was taken. Exploratory data analysis included an appraisal for spurious and extreme values and generating tables of summary statistics and distributional plots.

Table 1: Questionnaire used in a study of 20 New Zealand dairy sheep farms in the 2022-2023 season.

Category	Question
General farm information	
	Effective farm area (ha)
	Milking flock size at peak
	Number of seasons in operation (including 22/23)
	Total staff employed (including part time) - spring
	Total staff employed (including part time) - after spring
	Milking staff (including part time) - spring
	Milking staff (including part time) - after spring
	Number of hoggets
	Number of two-tooths
	Number of mixed age ewes
	Breed composition
	Lambing start date - hoggets
	Lambing start date - mixed age ewes
	When lambs were removed from ewes (age/weight)
	Is colostrum milked and fed to lambs?
	Did lambing happen on the same paddocks as last season?
	Were lambs taken off ewes with ≥3 lambs?
	Did lambing occur indoors or outdoors?
Milk production	
	Average milk production per ewe previous season (L/ewe)
	Average milk production per ha previous season (L/ha)
	What was the milk used for/where is it sent to?
	Over what period were ewes milked?
Milk harvesting	
	Use of gloves in milking shed (Y/N/personal choice/other)
	Is teat spray used for at least some of the season? (Y/N)
	What part of the season is teat spray used?
	Frequency of teat spray application (when used)
	Teat spray active ingredient
	Milking frequency
	Type of milking parlour (rotary, herringbone)
	Number of animal bails/positions in the parlour
	Number of available milking units
	Are there automatic cup removers?
	Are there automatic teat sprayers?
	Are there in line milk meters?
	What information do the meters collect (if applicable)?
	System pulsation frequency (cycles/min)

Category	Question
	System vacuum (kPa)
	Type of flow line (high/low)
	Temperature in the milk tank
	Is there an automated feeding system in the parlour?
	Type of feed troughs available (individual/group)
Animal health	
	Flock selenium status (if known)
	Selenium supplementation method (if applicable)
	List vaccines administered in last 12 months
	Are any on-farm microbiology tools used?

The amount of land dedicated to dairy sheep was not definite on two farms because it expanded and contracted in relation to other land uses on the same property within a season; in these cases the midpoint area was taken. The number of ewes per ha was calculated by dividing the peak number of ewes milked by the farm area. Staff numbers were defined as the maximum number of staff employed on a given day in the season, including part-time and casual staff. Multiple casual staff sharing the same role non-simultaneously were counted as a single staff member. Because many farmers employed more staff early in the season, staff numbers were presented for two time periods: “early” (during the lambing period and training of ewes in the milking parlour), and “mid to late” (the rest of the season). All part-time staff were assumed to be 0.5 full time equivalents (FTE). Age structure was calculated by recording the number of hoggets (ewes that were one year of age at last lambing), two-tooths (ewes that were two years of age at last lambing), and mixed age (MA) ewes (all older ewes). If the number of hoggets and two-tooths were known but not the number of MA ewes, the number of MA ewes was calculated as the difference between the peak number of ewes being milked and the number of hoggets and two-tooths. Breed data comprised the predominant breeds in each flock as reported by the farmers during the interviews, with breeds recorded as present or not. Flock breed proportions could not be calculated due to the limited data

available, the often rapidly changing breed compositions of the study flocks and the large amount of cross-breeding.

Milk production was estimated by the farmers who had sufficient information (volume of milk sold to a processor or processed on farm) by dividing the total volume harvested for commercial purposes by the total number of ewes milked. Data were restricted to milk harvested and sold and excluded milk suckled by lambs or harvested but not sold (e.g., transition milk fed to lambs). Colostrum was defined as the first secretion from ewes that had not been suckled or milked, and transition milk was defined subsequent secretions that were harvested within the first four days postpartum. Milk systems were defined as either high line or low line, with a high line system having the main milk line mounted above the ewes' udders.

Selenium supplementation methods were categorised into 1) added to feed (e.g., proprietary supplements mixed with concentrate feed), 2) selenised anthelmintics, 3) injectable products containing selenium alone or in combination with vitamin B_{12}, 4) selenised vaccines, 5) salt blocks placed in the paddock for *ad libitum* access, and 6) pasture application (e.g., selenium prills, seaweed fertiliser with added selenium). A vaccine was deemed to be used if it was administered to any age group (e.g., a farm was considered to use Toxoplasma vaccination if ewe lambs were vaccinated).

2.4 Results

2.4.1 General farm characteristics

Milk from the 12 North Island farms that sent all milk to a processor was made into milk powder products, while milk from the four South Island farms that sent all milk to a processor was used to make a mixture of cheese, yoghurt and fresh milk. The single farm that both processed its own milk and sent some to a processor sold fresh and flavoured

pasteurised milk to retailers, while the milk sent to a processor was made into milk powder products. Of the remaining three farms, one made cheese on farm, one sent milk to a cheese maker, and one sent some milk to a cheese maker and froze some for making yoghurt.

All farms lambed entirely in the spring except for the farm that sold fresh milk to retailers, which had an autumn-lambing flock in addition to a larger spring-lambing flock. There was a wide range in effective farm size, peak number of ewes milked, peak number of ewes milked per ha, and number of seasons the farms had been operating (Table 2). On 2/20 farms, later-lambing hoggets (farm P) or ewes from the conventional (non-milking) flock (farm T) were brought in after some ewes had already been dried off, so the peak number milked did not represent the total number milked. All ewes lambed outdoors on 17 farms. One farm lambed hoggets and ewes bearing three or more lambs indoors, and two farms brought some ewes indoors during inclement weather (typically ewes bearing three more more lambs).

Table 2: General characteristics of 20 New Zealand dairy sheep farms in the 2022-2023 season.

Variable	N farms with data	Median (range)
Farm area (ha)	20	50 (11-160)
Peak number of ewes milked	20	790 (171-1,530)
Peak n milking ewes/ha	20	15 (6-22)
Number of seasons the farm has operated (including 22/23 season)	20	4 (2-25)
Total number of staff (spring)	20	3 (2-10)
Total number of staff (after spring)	20	3 (1-10)
Number of milking staff (spring)	20	2 (1-4)
Number of milking staff (after spring)	20	2 (1-4)
Number ewes per staff member (spring)	20	244 (38-400)
Number ewes per staff member (after spring)	20	274 (38-400)
Percentage of flock that are hoggets	15	36 (10-41)
Percentage of flock that are two-tooths	15	29 (11-36)
Percentage of flock that are mixed age	15	40 (25-70)

2.4.1.1 Staff

The number of staff employed did not increase consistently with flock size (Figure 2). All except two farms employed the same number of staff during the spring and after spring, both in total and for milking. Farms J and Q employed more staff in total during the spring than after spring, and farms D and L employed more milking staff during the spring than after spring.

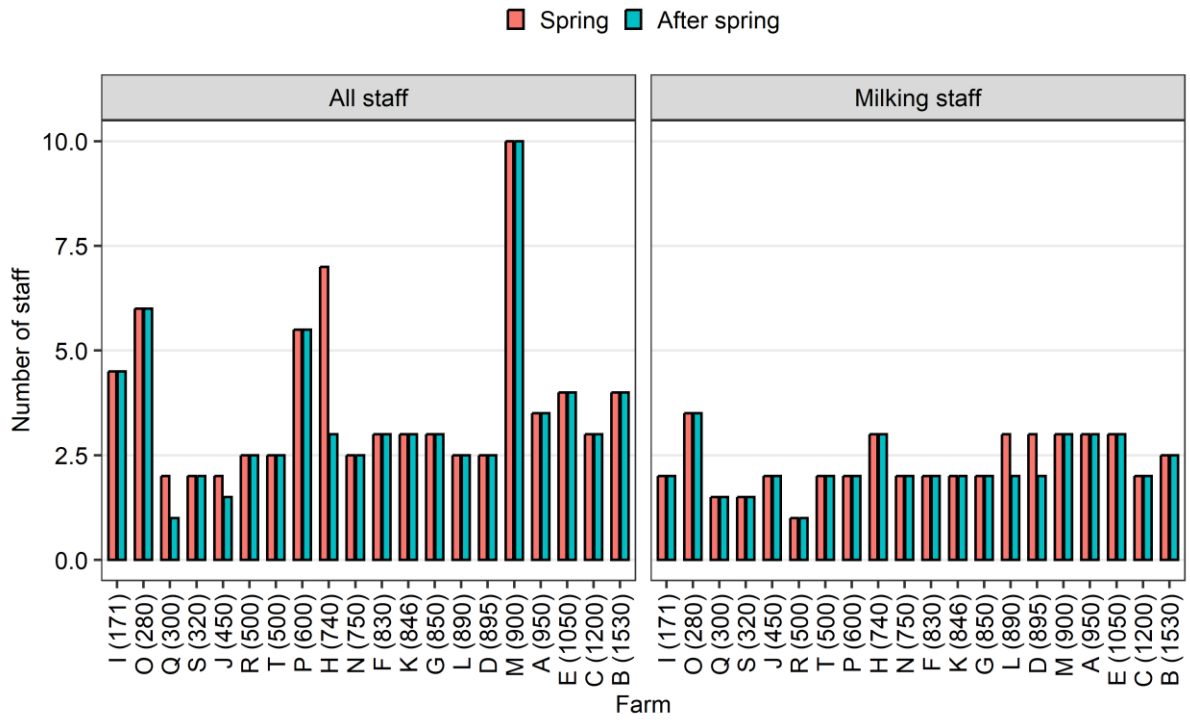

Figure 2: Staff numbers in a study of 20 New Zealand dairy sheep farms in the 2022-2023 season. Numbers in brackets are peak milking flock size, and farms are ordered left to right from smallest to largest peak milking flock size.

2.4.1.2 Ewe age and breed

Flock age structure was unknown on five farms due to not having detailed ewe-level records. On the 15 farms with data, there was a large variation across farms in the proportions of each age group (Table 2).

The following breeds were confirmed to comprise at least some of the genetics among the study farms (number of farms in brackets): Awassi (n=3), Coopworth (n=6), Dairymeade (n=1), Dorper (n=1), East Friesian (n=13), Highlander (n=2), Lacaune (n=10), Manech tête rousse (n=1), Poll Dorset (n=3), Southern Cross (n=7), Texel (n=1), Wiltshire (n=1) and Zealandia (n=5).

2.4.1.3 Lambing management

Lambing management is summarised in Table 3. Approximately half of the farms lambed the ewes in the same paddocks as the previous season. On two farms, pregnant ewes carrying three or more fetuses were brought indoors to lamb during inclement weather, while one farmer routinely brought all ewes carrying three or more fetuses indoors for lambing regardless of weather. All farms lambed hoggets later than mixed age ewes. Lambing start dates ranged from 25 July to 03 October (median = 18 August) for hoggets, and from 02 July to 10 September (median = 23 July) for mixed age ewes. Lambing dates were generally, but not always, later in southern farms than northern farms (Figure 3).

Table 3: Lambing management on 20 New Zealand dairy sheep farms in the 2022-2023 season.

Variable	Median (range)
Did the ewes lamb in same paddocks as the previous season?	
Yes	9 (45%)
No	11 (55%)
Did the ewes lamb indoors or outdoors?	
All outdoors	17 (85%)
All indoors	0 (0%)
Selected ewes lamb indoors	3 (15%)
Were lambs taken off ewes with ≥3 lambs?	
Yes	14 (70%)
No	6 (30%)
Was colostrum harvested and fed to lambs?	
Yes	15 (75%)
No	5 (25%)

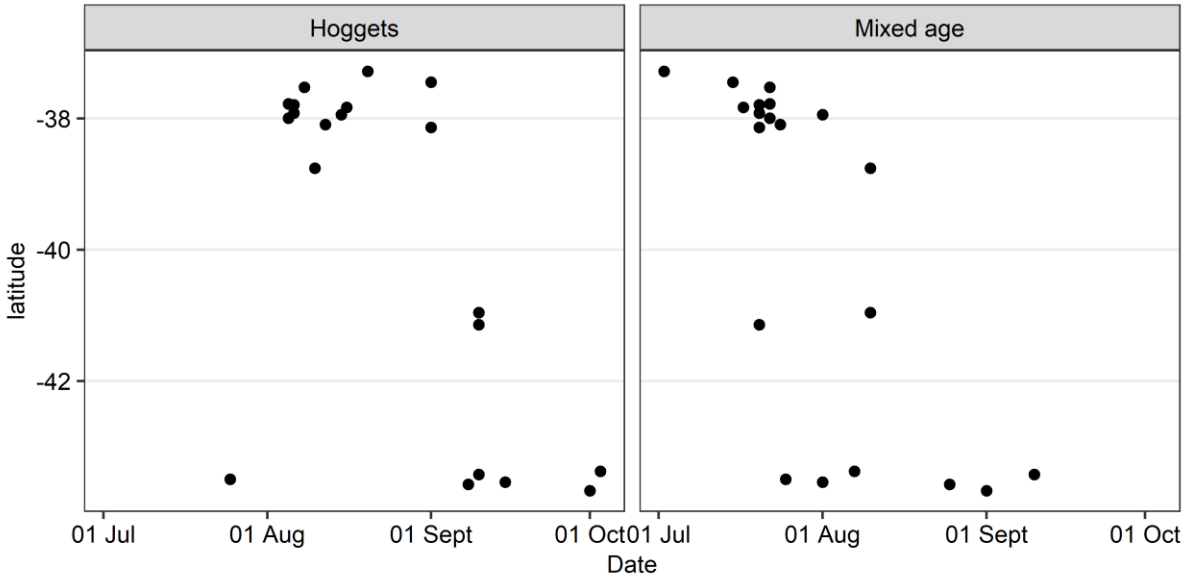

Figure 3: Lambing dates in a study of 20 New Zealand dairy sheep farms in the 2022-2023 season.

The timing of removal of lambs from ewes was highly variable, both between and within farms. Several farmers changed their policy within or between seasons to adapt to resource availability or adverse weather, or in response to trial and error. On 14 farms, lambs were mostly removed ≤10 days after birth and reared artificially, while on six farms most or all lambs were reared by the ewes and removed at a target age or weight.

All 14 farms that removed lambs ≤10 days after birth supplied milk to a processor. The lambs were removed at a median (range) of 2 (1 - 7) days when following their routine procedures. However, on nine of these farms, the timing of lamb removal was changed for some subsets of lambs. One farm (D) initially tried to remove lambs at four days but reduced it to a routine of one day due to lamb losses during wet weather at the start of lambing Two farms (O and K) removed lambs at 1.5 and 2 days, respectively, but left some lambs on the ewe for longer if they were subjectively deemed to need more time with the ewe. Another farm (N) brought lambs in at 2.5 - 3 days as a rule, but brought them in earlier during inclement weather. On four farms, the timing of lamb removal was delayed in the later part of the lambing period,

with one farm (R) not removing lambs born from hoggets until December (at four to five months of age) due to a shortage of lamb rearing staff, one farm (K) closing the lamb rearing unit in mid-September and rearing the remaining lambs on the ewe, one farm (B) removing lambs at two days but delaying this if the lamb barn was full or staff resources were limiting, and one farm (H) leaving lambs from hoggets on the ewe once the lambing shed was full. Another farm (L) reared 10% of lambs on the ewe as an experiment.

Of the six farms (F, I, P, Q, S, T) that removed lambs >10 days after birth, two supplied milk to a processor and four processed their own milk (one of which also sold some milk to a processor). The lambs were removed at a set age on one farm (F, 40 days) but for the other five farms, they were removed once they reached a target weight, or when removal fit in with other farm activities. One farmer (S) removed lambs when they reached 15 kg or six weeks of age, whichever came first, and one (Q) removed lambs once they reached 15 kg but only after 150 ewes had lambed (approximately 50% of the flock), so the lambs born earlier in the season were left on the ewe for longer and were therefore >15 kg at removal. Another farm (P) removed approximately one third of the lambs <10 days after birth but removed the rest once they exceeded 14 kg, at approximately five weeks of age. Lambs were typically removed on one farm (I) at four weeks, but this was delayed to approximately eight weeks in the study season due to flooding. On the farm (T) that milked ewes that were selected from a larger non-dairy flock, the lambs were removed at weaning (October to December) for slaughter based on weight, and milk harvesting commenced at that point.

Timing of lamb removal also depended on litter size on 14 farms. These farmers reported that they removed one or more lambs from ewes with triplets or quadruplets and reared them artificially. On 4/14 farms, this was done routinely, though one removed all quadruplets but left triplets on the ewe unless it was subjectively deemed necessary to remove them, and one removed one lamb from triplet and quadruplet litters after 10-14 days. On the other 10 farms,

lambs were only removed when judged to be necessary. When specific reasons were articulated, they included imbalance in lamb size, lambs judged to be underfed or mismothered, the ewe was unhealthy or at risk of becoming unhealthy, or the weather was inclement or likely to become so.

2.4.1.4 Colostrum and transition milk harvesting and feeding

Transition milk and, occasionally, colostrum, was harvested to varying extents on 15/20 farms. On the six farms that reared lambs mostly on the ewe (i.e., removed lambs >10 days after birth), three harvested some colostrum or transition milk. One farm removed lambs from a subset of ewes that were milked within four days of lambing but noted that transition milk blocked the milk feeding system. Colostrum or transition milk from ewes whose lambs died was harvested and fed to orphan lambs on one farm. Transition milk was hand milked from some ewes and stored on one farm. One of the three farms that did not harvest colostrum or transition milk sourced bovine colostrum for orphan lambs. On the 14 farms that removed lambs ≤10 days after birth, 13 harvested at least some transition milk: day one milk was harvested from ewes whose lambs died and fed to new lambs on two farms; while the remaining farms fed any transition milk collected in the first four days to lambs. One of these farms did not send milk for processing in the first two weeks of the season but stored it at approximately 1°C and fed it to lambs until exhausted. The farmer who did not harvest transition milk stated that it caused blockages in the automatic feeders.

2.4.2 Milk production

The period over which farmers milked ewes for commercial purposes varied widely depending on timing of lamb separation, the time period over which processors purchased milk, weather events, and farmer choice (Figure 4). For the 12 North Island farms that supplied milk solely to a processor, the first collection date varied between 03 August and 31

August (median = 04 August) and the last collection date varied between 22 March and 05 May (median = 20 April). This corresponded to season durations of 221 to 274 (median = 259.5) days. Detailed information was not available for the other eight farms. Among these, the two North Island farms that did not supply processors commenced milking six and eight weeks after lambing and continued to May and February, respectively (resulting in season durations of nine and five months, respectively). On the latter farm, ewes were dried off earlier than normal because severe spring floods caused a delay in the commencement of milk harvesting and therefore a reduction in the productivity of the flock for the rest of the season. For the two South Island farms that started harvesting milk ≤10 days after lambing, one milked from July to May and the other from August to March, corresponding to respective durations of 11 and eight months. For the four South Island farms that started harvesting milk >10 days after lambing, milk collection on each farm occurred between August and February (7 months), November and February (3 months), December and February (3 months) and December and March (4 months). On three of those farms, the owners stated that, in general, the length of the season is variable and depends on ewes' productivity and how it fits in with other operations and resources on or off the farm.

Estimated mean season milk volume per ewe and per ha, and the number of days across which milk was collected for commercial purposes, are summarised in Table 4. Milk volumes were not recorded or could not be calculated due uncertainty caused by the varying numbers of ewes being milked across the season on 7/20 farms. All three statistics varied widely across farms.

Table 4: Milk production statistics of 20 New Zealand dairy sheep farms in the 2022-2023 season. Milk production was not recorded on seven farms.

Variable	N farms with data	Median (range)
Mean season milk production per ewe (L)	13	261 (210-400)
Mean season milk production per ha (L/ha)	13	4.33 (2.11-8.87)
Number of days milk was harvested for commercial purposes	20	244 (81-297)

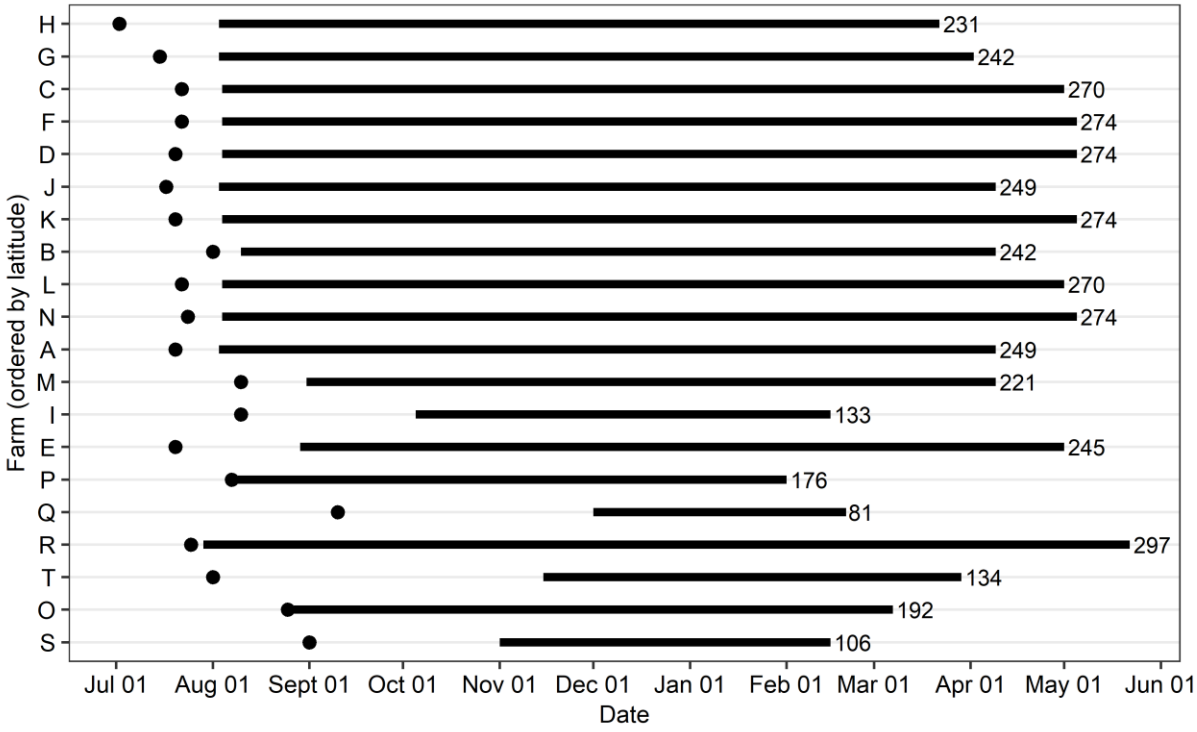

Figure 4: Timing of lambing onset (points) and commercial milk harvesting (lines) on 20 New Zealand dairy sheep farms in the 2022–2023 season. Numbers represent the milk harvesting period length (days).

2.5 Milk harvesting

Parlour design, milking plant specifications, and milking procedures are summarised in Tables 5 and 6. Nine of the 15 farms that milked twice daily at the start of the season changed to once daily milking later in the season. Herringbone parlours were the most common design (15/20 farms), and 10/20 parlours had a high line system. The median number of milking units was 41, with medians (ranges) of 42 (16 - 60) and 35 (16 - 70) for herringbone and rotary parlours, respectively. System pressure varied from 32 to 43 kPa. Median (range) pressures were 38.25 (37 - 42) and 38 (32 - 43) for farms with high and low line systems, respectively.

Table 5: Parlour design and milking procedures on 20 New Zealand dairy sheep farms in the 2022-2023 season.

Variable	N (%)
Milking frequency	
Twice daily	6/20 (30%)
Twice daily for part of the season then once daily	9/20 (45%)
Once daily	5/20 (25%)
Milking parlour type	
Herringbone	15/20 (75%)
Rotary	5/20 (25%)
Type of flow line (high/low)	
High	10/20 (50%)
Low	10/20 (50%)
Inline milk meters	5/20 (25%)
Information collected by inline milk meters	
Milk volume	4/5 (80%)
Milk volume, fat and protein	1/5 (20%)
Not applicable	15
Automatic cup removers	7/20 (35%)
Automatic teat sprayers	0/20 (0%)
Automatic feeding system	15/20 (75%)
Type of feed trough in parlour	
Group	1/20 (5.0%)
Individual	15/20 (75%)
None	4/20 (20%)
Use of gloves during milking	
Compulsory/all	8/20 (40%)
Optional/some	11/20 (55%)
Not worn	1/20 (5.0%)
Teat spray use	
All season	8/20 (40%)
Part season	10/20 (50%)
Not used	2/20 (10%)
Frequency of teat spray application	
Every milking	18/18 (100%)
Not applicable	2
Teat spray type	
Chlorhexidine	13/18 (72%)
Iodine	5/18 (28%)
Not applicable	2

Table 6: Plant vacuum and pulsation, target bulk milk temperature, number of milking units and number of flock milk recording tests on 20 New Zealand dairy sheep farms in the 2022-2023 season.

Variable	Median (range)
System pressure (kPa)	38.0 (32-43)
Unknown	1
Pulsation frequency	130.0 (119-182)
Unknown	3
Bulk milk target temperature (°C)	2.5 (1-4)
Number of milking units in the parlour	41 (16-70)
Number of flock milk recording tests in the 22-23 season	0 (0-6)

Automatic in-parlour feeding systems were installed on 15/20 farms and feed was manually dispensed on one farm, with most systems (15/16) delivering feed into individual feed bins. Automatic cup removers were installed on 7/20 farms, inline milk meters on 2/20 farms, while no farms had automatic teat sprayers. Milk volume was the only measurement made by inline milk meters on 4/5 of the farms with inline meters, while the meters also recorded milk fat and protein on 1/5 farms. Glove use during milking was variable, with 8/20 (40%) of farms mandating the use of gloves. Teat spray was used all season on 8/20, part of the season on 10/20, and not used on 2/20 farms. When in use, teat spray was applied at every milking. Teat spray active ingredients were either chlorhexidine or iodine, with chlorhexidine-based products being more common (13/18 farms). Flock milk recording was performed on 7/20 (35%) farms, with one farm performing a single test, two farms performing three tests, three farms performing four tests, and one farm performing six tests. The timing of the tests was not recorded.

2.5.1 Flock selenium management and vaccination practices

Milking flock selenium status was unknown on 12/20 farms, though one of these farms performed some testing of non-dairy ewes on the same property, and another collected liver biopsy data from lambs at slaughter. For the eight farms that had information on flock

selenium status, it was based on blood samples of ewes on seven farms and liver biopsies as well as blood samples of ewes on one farm. The farmers reported that selenium was adequate on all eight farms that collected samples from milking ewes, adequate on the farm that used liver biopsy data from lambs at slaughter, and low on the farm that collected samples from non-dairy ewes.

Selenium was supplemented on 19/20 farms, with the one non-supplementing farm not having information on its selenium status. Of the six different methods used, in-feed supplementation was the most common (10/20 farms, Table 7).

Table 7: Selenium supplementation methods used on 20 New Zealand dairy sheep farms in the 2022–2023 season (milking flock only).

	Selenium supplementation method						
Farm	**Added to feed**	**Selenised anthelmintics**	**Injectable product**	**Selenised vaccines**	**Salt blocks**	**Applied to pasture (prills/fertiliser)**	**None**
A							✓
B				✓			
C	✓		✓				
D	✓	✓					
E						✓	
F	✓						
G	✓						
H					✓		
I	✓		✓				
J			✓			✓	
K	✓		✓				
L	✓	✓	✓				
M	✓		✓				
N	✓						
O		✓			✓		
P		✓		✓	✓		
Q		✓		✓			
R			✓				
S	✓	✓					
T		✓		✓		✓	
Total	**10**	**7**	**7**	**4**	**3**	**3**	**1**

All farms used clostridial vaccines, and 15/20 vaccinated at least some animals against leptospirosis (Table 8). All 15 farms that used leptospiral vaccines vaccinated the milking ewe flock prior to lambing, though on one farm ewes were only vaccinated in seasons when staff were employed (i.e., when the owner was the sole worker, the ewes were not vaccinated).

Table 8: Vaccines used on 20 NZ dairy sheep farms in the last 12 months (2022–2023).

	Vaccines used						
Farm	**Clostridia**	***Toxoplasma***	***Campylobacter***	***Leptospira***	**Scabby mouth**	***Salmonella***	**Caseous lymphadenitis**
A	✓	✓	✓	✓	✓	✓	
B	✓	✓	✓		✓		
C	✓	✓		✓	✓	✓	
D	✓	✓	✓	✓	✓	✓	
E	✓	✓	✓	✓			
F	✓	✓	✓	✓	✓	✓	
G	✓			✓			
H	✓	✓	✓	✓	✓		
I	✓	✓	✓	✓			
J	✓	✓	✓	✓	✓		
K	✓	✓	✓	✓	✓		
L	✓	✓	✓	✓	✓	✓	
M	✓	✓	✓				
N	✓	✓	✓	✓	✓	✓	
O	✓				✓		
P	✓	✓	✓	✓			
Q	✓	✓	✓	✓	✓		✓
R	✓			✓			
S	✓	✓	✓				
T	✓	✓	✓			✓	
Total	**20**	**17**	**16**	**15**	**12**	**7**	**1**

On-farm microbiology tools were used for confirming mastitis diagnoses and/or the bacterial causes on three farms, consisting of the Mastatest system (Mastatest Ltd., Hamilton, New

Zealand) on two farms and the Farm Medix system (Farm Medix Ltd., Hamilton, New Zealand) on one farm.

2.6 Discussion

This study provides, to our knowledge, the first systematic, industry-wide description of a large number of commercial sheep milking farms in New Zealand. Approximately half of the farms operating in the 2022-2023 season participated, yielding a consistently collected cross section of the industry at that time. This serves as a baseline for benchmarking and development. Despite all farms sharing the core features of lambing entirely or predominantly in the spring, managing ewes predominantly or entirely outdoors, lambing hoggets approximately four weeks later than older ewes, supplementing selenium (except one farm), and administering clostridial vaccines, there was substantial diversity between farms. This was particularly evident for farm scale, lambing and lamb management, season length, and data recording. Different New Zealand sheep milking farms should therefore be regarded as unique and adaptive rather than as a single "pasture-based" system. The median of four seasons in operation demonstrates the relative youth of the New Zealand industry and may explain the diversity of farm practices, with a period of experimentation being required to establish practices and attributes that are optimised for New Zealand conditions.

How the farms operated appeared to be influenced by the farmers' priorities and objectives, as well as resource availability. This was reflected in the wide range in milk harvesting periods (81 to 297 days). Farmers who supplied milk to processors mostly separated lambs from ewes within 10 days of birth and the period over which milk was harvested was predetermined by lambing dates and processor collection. On the other hand, all farmers who processed their own milk separated lambs at a later date and the milk harvesting period was more flexible. Sheep milking was the primary purpose of most farms, but some farms ran

milking operations within the context of a wider conventional (non-dairy) sheep farm that dictated some decisions, and one farm even supplemented the milking flock with more ewes from the conventional flock when needed. Resources, such as staff and facility availability, and farmer priorities affected some practices like lamb rearing and the timing of milk harvesting.

Staff numbers did not increase consistently with flock size and did not change between spring and the mid-late season on most farms, implying that the number of ewes per labour unit generally increased with farm size and heavy workload during spring. The median ratio of ewes to staff members was 244 during the spring and 274 for the rest of the season. These values are close to the average ratio of 244 ewes per labour unit among technically efficient semi-extensive French sheep milking farms (Theodoridis *et al.* 2022). Some automation was apparent, with in-shed (i.e., the milking parlour) feed dispensing and automatic cup removers being used on 75% and 35% of farms respectively, though no farms used automatic teat sprayers. Further work is needed to determine if the farmers would prefer to employ more staff but are limited by economic constraints, staff availability or staff training, and to clarify the value of automation.

Lambing and lamb removal practices varied widely between farms, and policies often changed within the season in response to resources, conditions and experimentation. Early lamb separation extends the milk harvesting period and supply of milk. Most farmers separated lambs within 10 days of birth and reared them artificially, with the rest rearing the lambs on the ewe and separating the lambs at a target age or weight, at which point milk harvesting commenced. None of the farms practiced a mixed system, in which lambs were separated during the day, and ewes were milked once or twice daily. New Zealand research showed that a mixed system did not negatively impact lamb growth, health, weaning age and post-weaning mammary development compared to leaving the ewes and lambs together and

commencing milk harvesting later at separation, and allowed for more milk to be harvested from the ewes, but also acknowledged the added complexity of managing such a system (McCoard *et al.* 2023a). Many farms applied different lamb removal practices for different groups or at different times of the season, emphasising the diversity and adaptive nature of management on New Zealand sheep milking farms. Removal of lambs and commencement of ewe milking at 24 hours postpartum was shown to increase whole-season milk yield among East Friesian crossbred ewes in the United States (McKusick *et al.* 2001). However, while not statistically significant (p reported as between 0.05 and 0.10), separating lambs and ewes (and starting machine milking of the ewes) at 30 days postpartum may result in faster lamb growth: mean (SEM) weights at 120 days of age were 43.7 (1.2), 45.9 (1.8) and 47.3 (1.6) kg for lambs removed at 24 hours postpartum, lambs that suckled their dams for 9 hours/day until weaning, and lambs that suckled exclusively until weaning, respectively, in the study of McKusick *et al*. (2001). Furthermore, artificially rearing lambs is labour intensive. The timing of lamb removal in New Zealand is therefore a choice that trades off between the value of extra milk sold and the costs of labour and potentially reduced lamb growth rates, though use of automatic lamb feeding systems can ease that demand.

It was common for farmers to identify lambs or ewes that they deemed were at risk of poor health during the lambing period, and to rear those lambs artificially or bring the ewes indoors. Several farmers had access to housing for these lambs and ewes, and brought ewes in proactively during inclement weather. We did not investigate the effectiveness of these interventions. Providing shelter is likely to be beneficial to ewe and lamb health, but housing of ewes and artificial lamb rearing increase resource and labour costs and are reliant on the skills and availability of staff and appropriate infrastructure.

Colostrum management is a critical part of dairy cow and calf management, but it is not clear if failure of transfer of passive immunity via colostrum is common on New Zealand dairy

sheep farms. Colostrum or transition milk was collected to varying extents on 75% of the farms for feeding to orphan lambs or lambs that were artificially reared. Some colostrum or transition milk was collected from ewes on half of the farms that reared lambs on the ewes for >10 days. For the farms that removed lambs and started harvesting milk within 10 days of lambing, some colostrum or transition milk was collected, but on 12/14 of these farms, it was in fact transition milk collected before day five after lambing. Colostrum or transition milk was reported to block automatic lamb feeding machines by two farmers, implying a practical constraint that could reduce colostrum feeding under labour pressure.

Lactation length and yield varied widely, though yield estimates were often derived from bulk-milk volumes rather than individual ewe records, so comparisons should be made cautiously. The median milk-harvesting period of 244 days is expected to overestimate the median ewe lactation length, since not all ewes had milk harvested from the start to the end of the harvesting period. Data are limited on lactation length for dairy ewes, but Hernandez *et al.* (2012) reported a mean of 238 days for intensively managed ewes on a single Spanish farm. On several farms, milk harvesting commenced only after lambs were partially or fully reared on the ewe, resulting in shorter effective milking periods and lower harvested yields by design.

Most of the milking facilities were <10 years old. Herringbone designs were dominant, and most parlours had feeding systems. Vouraki *et al*. (2018) collected data from 28 semi-intensive Greek farms and found modal pressure and pulsation frequency to be 40 kPa and 150 cycles/minute, respectively. I.e., although the pressure was similar to the farms in the present study, the pulsation frequency was higher on the Greek farms. Pulsation frequency varied widely on our study farms (range = 119-182). Peris *et al*. (2003) compared udder health, teat end oedema and somatic cell counts of ewes milked with pulsation frequencies of 120 or 180 cycles/minute and found no difference between the two frequencies. It is not clear

what the optimal pulsation frequency is for dairy ewes, and it may depend on the physical characteristics of the ewes and their teats. Although higher pulsation frequencies are commonly used in dairy sheep than in dairy cows, the biological basis for this is not well established; calf sucking rates of approximately 113–133 sucks/min have been reported (Braun *et al*. 2022), while comparable data for lambs are less readily available because sheep studies often describe suckling bout frequency and duration rather than individual sucks per minute. System pressure and pulsation frequency were not found to be risk factors for subclinical mastitis among these 20 farms (Chambers *et al.* 2025b), but this finding should be interpreted cautiously because the farm-level analysis had limited power.

Hygiene practices also varied. Use of gloves was compulsory on only 40% of the farms, and only 40% of farms used teat spray all season. To our knowledge, there is no published research on whether wearing gloves or post-milking teat disinfection reduces the risk of intramammary infection on dairy sheep farms, but use of gloves was associated with a lower risk of subclinical mastitis among dairy cows in Australia (Plozza *et al.* 2011), and post-milking teat disinfection has been shown to reduce the incidence of intramammary infections among New Zealand dairy cows (Williamson and Lacy-Hulbert 2013). Nevertheless, neither glove usage nor teat spray policy were confirmed as risk factors for subclinical mastitis among these 20 farms (Chambers *et al.* 2025b), but again, these findings should be interpreted cautiously because the farm-level analysis had limited power. Post-milking teat disinfection was not found to affect the risk of subclinical mastitis in dairy sheep by Michael *et al*. (2023). Given the labour costs of manual post-milking disinfection, there is a case to evaluate standard operating procedures and the feasibility and performance of automation for teat disinfection in sheep parlours.

Selenium status and supplementation data were collected to investigate mastitis risk factors because an association has been found between low blood selenium concentration and

clinical mastitis incidence in dairy sheep (Giadinis *et al.* 2011), and New Zealand soils are known to be naturally low in selenium (Ellison 2002). Indeed, almost all (19/20) farms in the present study provided supplemental selenium. However, this supplementation was not linked to evidence of deficiency, with selenium status being unknown on 60% of the farms, and not deficient on 7/8 farms that performed testing on dairy (7 farms) or non-dairy ewes (1 farm). This highlights the potential for unnecessary supplementation on many of these farms as well as insufficient supplementation on farms where deficiency is a problem. Wider uptake of routine periodic testing of milking ewes is needed, and the value of bulk milk testing should be explored.

While some form of clostridial vaccination was ubiquitous, only 75% of farms used a leptospiral vaccine. In New Zealand, leptospirosis is an important occupational zoonosis and leptospiral vaccination is deeply embedded in the dairy-cattle sector, with a survey of 200 randomly selected dairy farms finding vaccination programmes on 199 farms (Yupiana *et al*. 2021). Despite this high vaccine uptake, 26.5% of farms had at least one cow shedding pathogenic *Leptospira* in urine, and the presence of sheep was associated with increased odds of shedding (Yupiana *et al*. 2020). By comparison, the 75% uptake observed here suggests that leptospirosis risk management may be less consistently implemented in dairy sheep than in New Zealand dairy cattle. A study of non-dairy sheep carcasses at a New Zealand abattoir found that 31% of the sheep were positive on urine leptospiral qPCR, 29% on kidney qPCR, and 57% were seropositive (Fang *et al.* 2015). In New Zealand dairy cows, The prevalence of leptospirosis among dairy ewes has not been studied in New Zealand to our knowledge but, given the prevalence among non-dairy ewes and the more regular contact staff have with dairy ewes, structured leptospirosis risk management, including targeted surveillance in dairy ewes, is warranted. At the time of the study, no mastitis vaccines were registered for use in

sheep in New Zealand; consequently, the questionnaire focused on vaccines that were available and relevant to routine flock health management.

The New Zealand sheep-milking industry has a large opportunity to increase data collection. While technologies such as inline metering systems and flock management software exist and have been developed for dairy sheep, their uptake in commercial sheep dairy farms remains limited in New Zealand. Ewe-level data, including breed, lactation length, and milk yield, were sparse on many farms, with milk recording conducted on only seven farms and limited to one to six events per season. The rare use of milk recording in this study suggests that barriers such as hardware cost, cost–benefit uncertainty, data management and interpretation challenges, staff training requirements, and uncertainty about long-term value constrain adoption. Qualitative research to identify the barriers is needed. Addressing these barriers through demonstration of value, staff training support and demonstration of its use on commercial farms could help accelerate routine data collection and improve benchmarking, genetic evaluation, and precision management.

Our study covered approximately 50% of the industry in the 2022-2023 season and used a single-interviewer protocol with a standardised questionnaire, but it also had limitations. Variation in climate, changing management trends, and random variation could cause the extent and characteristics of mastitis to change across time. Much of the data was based on farmer estimates and recall, the farms were purposively selected (not randomly), the study ran over a single season, and ewe-level data were limiting. These factors preclude causal inference and limit the baseline to the 2022–2023 season, but the study nevertheless provides a useful reference point for future comparisons as the industry develops.

Practically, the findings support: 1) seasonal staffing plans and training focused on the busy spring period; 2) evaluation and standardisation of milking hygiene SOPs (glove use, teat disinfection) with attention to labour impacts and return on investment; 3) development of New Zealand-specific milking-plant guidance for sheep; 4) periodic trace element status testing; and 5) a strategy to collect data, prioritising simple, decision-oriented recording. While some of these topics have been addressed in previous New Zealand and international studies, important uncertainties remain under contemporary New Zealand pasture-based sheep dairy conditions. Priority research questions include the productivity, welfare, and labour economics of early versus later lamb separation under New Zealand conditions; how to attract and train staff and common training needs; the performance and animal health consequences of alternative milking plant parameters; trade-offs with implementing automation; the prevalence of leptospirosis among grazing dairy ewes; and interventions to increase adoption and utility of ewe-level recording. Several of these practices are also shaped by milk processor requirements related to milk quality and contractual obligations, which may constrain on-farm decision making and should be considered in future research.

2.7 Conclusions

New Zealand dairy sheep farms were relatively young and highly diverse, particularly in terms of lambing and lamb management, the period of milk harvesting, and milk yield per ewe per season. Common practices included spring lambing, predominantly outdoor management, near-universal selenium supplementation, and universal clostridial vaccination. Staffing did not scale consistently with flock size, suggesting potential workload pressure during spring and a role for staffing plans, staff development and automation. Given wide variation and data constraints, yields and season lengths should be interpreted cautiously, but they indicate competitive potential under New Zealand conditions. Key opportunities for sector progress include strengthening ewe-level data capture, clarifying the benefits and

practicality of milking-hygiene interventions for sheep, and implementing evidence-based trace element and leptospirosis risk-management programmes. Together, these steps would support benchmarking, genetic and managerial gains, and improved animal health and productivity under New Zealand's predominantly pasture-based systems.

2.8 Acknowledgements

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3 TEAT AND UDDER MORPHOLOGY AND PATHOLOGY OF NEW ZEALAND DAIRY EWES

Having presented a profile of New Zealand dairy sheep farms in the previous chapter, this chapter lays further groundwork for the subsequent chapters on mastitis and milk quality by describing teat and udder conformation and pathology.

This builds on the first chapter and starts to focus on milk harvesting and mastitis by describing the mammary glands, whose conformation and health are critical to the topic of mastitis and milk quality. Chapter 2 highlighted the large variance between and even within farms in terms of management systems, and this chapter similarly highlights the large variation in udder conformation. It also provides a reference to farmers, veterinarians and other stakeholders on the importance of conformation, what to look for, how to score ewes, and where the opportunities lie to make improvements.

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Teat and udder morphology and pathology of New Zealand dairy ewes

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3.1 Abstract

Aims: To describe the morphology and the prevalence of pathology of the teats and udders of New Zealand dairy ewes.

Methods: A repeated cross-sectional study was conducted on 20 commercial New Zealand sheep milking farms over the 2022-2023 season. Approximately 15 ewes were examined on each farm in early, mid, and late lactation. The ewes were randomly selected at each visit. Four udder morphology measures were scored on a 5-point scale: depth (the distance between the udder cleft and the abdominal wall, with a score of 3 applied when the cleft was level with the hocks and a score of 5 when the cleft was against the belly), suspension (ratio of the width at the abdominal attachment to the height, with a score of 3 indicating a ratio of 1 and a score of 5 indicating an udder with an attachment width greater than the height), separation (the extent of the udder cleft, with a score of 1 equating to no separation and a score of 5 equating to the cleft extending more than half of the udder height), and teat placement (determined from the vertical distance between the teat attachments and the most distal point of the udder, with a score of 1 indicating teats that are placed at the most dependent part of the udder and oriented downward and a score of 5 indicating teats placed high up the udder and oriented laterally). Teat length and width were measured, and the presence of

supernumerary teats and asymmetry was recorded. Teat end hyperkeratosis was scored on a four-point scale. The presence of visible teat/udder inflammation and lesions was noted. Teats were palpated for consistency, thickening of the teat canal, and patency. Glands were palpated for consistency and the presence of nodules. Teat end hyperkeratosis, inflammation and lesions of the teats and glands, and teat and udder palpation data were converted to ewe-level scores. The presence of a non-lactating gland (a "half udder", subjectively judged to be producing a small volume of milk compared to the contralateral gland, or to be non-lactating) was recorded. Data were analysed using descriptive statistics.

Results: Across the three visits, 893 observations were made on 882 unique ewes. Mean (SD) teat length and width were 27.5 (4.9) and 15.8 (2.9) mm. Udder depth, separation, suspension, and teat placement had modal scores of 4, 3, 3, and 3 respectively, and varied across visits and age groups. The prevalence of udder asymmetry increased with age, with prevalences of 27% for hoggets (one-year-olds at lambing), 38% for two-tooths (two-year-olds), and 43% for mixed age ewes. Supernumerary teats were observed in 15% of ewes. There was between-farm variation in all udder morphology variables. Ewe-level prevalences of teat end hyperkeratosis (of any degree of severity), and gland/teat inflammation, lesions, palpable defects, and half udders were all <6%.

Conclusions: Morphology was like that observed in overseas dairy sheep. Teat dimensions, udder depth, separation, suspension, and teat placement, and presence of supernumerary teats varied between farms. Udder depth, separation and suspension scores decreased with age, while teat placement score and the prevalence of asymmetry increased with age. Teat and udder pathology was rare.

Clinical relevance: This is the first systematic study of teat and udder morphology and pathology in New Zealand dairy ewes. It provides baseline information that will be useful for

farmers and industry partners to compare their own flocks to the results in this dataset, identify areas for improvement, inform future studies, and compare future studies against.

Keywords: Ovine, dairy, morphology, pathology, teat, udder.

Abbreviations: BCS: body condition score; DIM: days in milk.

3.2 Introduction

The New Zealand dairy sheep industry has experienced rapid growth in the last decade, with an estimated 30 commercial farms milking approximately 30,000 ewes in 2022 (McCoard *et al.* 2023). Modern facilities and equipment are therefore commonplace and machine milking is standard. In the progression from start-up flocks with mixed breeds to consolidated flocks specialised for dairy farming, farmers may focus on teat and udder conformation because these heritable traits impact milkability (how efficiently and easily milk can be harvested from the ewe) and consequently productivity, teat and udder health, and milk quality (Fernandez *et al*. 1997, Sagi and Morag, 1974, Marshall *et al*. 2023).

The ideal dairy ewe allows milk to be harvested rapidly without manual assistance and has teats that are well-placed for easy application of milking machinery. Milk is stored in two compartments in the ovine udder: the alveolar and the cisternal compartments. In sheep, cisternal milk comprises 25-75% of total milk volume depending on the breed but is typically >50% in dairy breeds, which is higher than meat breeds (Rovai *et al.* 2004). Moreover, differences in cisternal volume but not alveolar volume have been found between high and low-producing ewes, suggesting that cistern volume is important for milk production (Rovai *et al.* 2008). The suspensory ligament creates the intermammary groove and therefore visible gland separation. It is also thought to affect the change in udder conformation as milk is drained, and therefore the positioning and angle of the teats during the milking process, and in turn the amount of milk that sits below the level of the teats. This is particularly important

in ewes, with the cistern comprising a relatively large proportion of milk volume. Furthermore, ewes with lax suspensory ligaments tend to develop horizontally oriented teats, making machine milking more challenging (McKusick 2000). Sagi and Morag (1974) demonstrated higher milk yields among machine-milked ewes with stronger gland definition and more downward-pointing teats. Ewes with taller cisterns (i.e., a greater udder volume below the level of the teats), while on average producing greater milk yields, also often require manual elevation of the udder to allow that milk to drain into the teats, which increases the time required to milk the ewe and the risk of overmilking (McKusick *et al.* 2003). New Zealand research found that higher udder depth scores (i.e., less pendulous udders), lower separation scores (poorly defined glands), higher attachment scores (udders that are relatively wide in relation to their height), and teats with a backwards angle were negatively associated with some or all of milk, fat, protein, and lactose yields (Marshall *et al.* 2023). Teat and udder morphology therefore clearly impact the productivity and milkability of dairy ewes.

Morphology is also important for udder health. Strong gland separation, well-attached udders, and higher udder depth scores (less pendulous udders) have been shown to be associated with lower somatic cell counts (Huntley *et al.* 2012; Marshall *et al.* 2023).

Teat and udder morphology are heritable and therefore responsive to selection, making selection for these characteristics an important tool for farmers. Fernandez *et al*. (1997) studied Churra breed ewes and determined heritabilities of 0.16, 0.17, 0.24, 0.18 and 0.24 for udder depth, udder attachment, teat placement, teat size, and udder shape, respectively. However, the same study showed that selection solely for milk yield, without attention to milkability, is likely to have a negative effect on udder conformation because higher milk yields were correlated with teats that were less vertically oriented. Furthermore, while more pendulous udders were positively correlated with milk yield, ewes with very pendulous

udders can be harder to milk by machine and carry a higher risk of mastitis (Huntley *et al.* 2012; Marshall *et al.* 2023).

Reliable and practical visual assessment of teat and udder morphology is thus important for genetic selection. Several different systems have been developed. Linear scoring systems have been recently developed, in which each trait is scored on a numerical scale. The original system used a 1-9 scale (Fuente *et al.* 1996). There are several variations of this system in use around the world, including a 1-9 scale used in New Zealand for dairy sheep by Marshall *et al*. (2023) and a 1-5 scale used for non-dairy sheep by Griffiths *et al*. (2019), both of which were influenced by the system developed by Casu *et al*. (2006).

Teat and udder pathology are important for milk quality and mastitis control. Teat end hyperkeratosis, defined as thickening of the teat canal and teat end skin, is a known risk factor for mastitis in dairy sheep (Vouraki *et al.* 2018). Other observable pathological changes, such as inflammation of the skin, lesions, lumps, and udder asymmetry, may be sequelae of mastitis or other conditions. The prevalence of palpable udder defects has been described in non-dairy sheep in New Zealand (Ridler *et al.* 2021; Zeleke *et al.* 2021; Marshall *et al.* 2023), but the prevalence of teat and udder pathology in New Zealand dairy sheep is unknown.

Given the importance of teat and udder morphology, the small amount of published morphology data and the lack of published pathology data for New Zealand dairy sheep, the objectives of this study were to describe teat and udder morphology and the prevalence of visible and palpable teat and udder pathology in New Zealand dairy ewes. Our purpose was to establish a baseline set of data that represents the New Zealand situation at this stage in the industry's development, to understand the variability between flocks, identify areas for improvement, and provide a reference point for future studies.

3.3 Materials and Methods

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

3.3.1 Study design, setting and participants

A repeated cross-sectional study was conducted on 20 commercial New Zealand sheep milking farms. The farms were selected to represent a range of locations and systems and were previously described in Chambers *et al*. (2024). Briefly, the farms were in the Waikato (n=12), Wairarapa (n=2), and Canterbury (n=6) regions. All farms lambed 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 three farms, which lambed some ewes indoors (e.g., 1-year-old ewe lambs and/or ewes bearing three or more lambs, either routinely or only during inclement weather).

Morphology/pathology assessments were planned on three occasions on each farm during the 2022/2023 lactation season, at times corresponding approximately to early, mid, and late lactation.

Initially, we aimed to select 30 ewes per visit but, after visiting three farms during visit 1, this was reduced to a minimum of 15 ewes because of concerns about the amount of time taken and the length of time the ewes were held off pasture. At the first visit, the ewes were examined prior to being milked during the morning milking on the first three farms. Thereafter, examinations occurred two to three hours after the morning milking to avoid prolonging milking time, except for one farm, where ewes were examined prior to milking at the morning milking at all three visits. The ewes were returned to the milking flock after examination.

All ewes that lambed in the 2022-2023 season and were being milked at the time of each visit were eligible. As this was part of a wider study, ewes were to be excluded for reasons unrelated to the present study: if they were 1) under treatment or had been treated within the previous 30 days for illness; 2) were diagnosed with clinical mastitis on the day of sampling (defined as visual or palpable udder changes with clots in the milk); 3) 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 and/or faeces that the operator deemed it unlikely that milk samples would be uncontaminated.

Ewes were randomly selected using a calculation based on the total number of ewes being milked at the time of the visit. For the first two farms at visit 1, to select 30 ewes, the number of ewes being milked was divided by 30 to calculate the number *p*. A random number generator was used to select the position of the first ewe to be selected (i.e., a whole number ranging between 1 and the number of milking positions in a rotary parlour or one side of a herringbone shed). Then every p^{th} ewe was selected and examined during milking. If a ewe was excluded, the adjacent ewe in the parlour was selected. For the third farm visited at visit 1, the same process was followed but the ewes were separated at the milking prior to the visit by the farmer. For all other visits, on the day prior to the visit, the number of ewes being milked was divided by 18 to calculate *p*, and the same process was followed as above. This provided 15 ewes and three spares in case of exclusions since these ewes were examined outside of milking time and adjacent ewes could not be selected. Ewes were presented to the research team and the first 15 eligible ewes were included. The farmers were responsible for selecting and separating ewes.

There were 18 known dairy sheep farms in New Zealand in 2019 (Anonymous 2020), and there were estimated to be approximately 40 commercial farms at the start of the 2022/2023 milking season. We enrolled 20 farms to achieve a study that included approximately 50% of

New Zealand dairy sheep farms. The sample size of the present study was determined by another part of the wider study on mastitis in dairy sheep and was based on being able to estimate the prevalence of a binary variable with an expected prevalence of 26% and an intraclass correlation coefficient (ICC) of 0.06. The original sample size of 30 ewes per farm (1,800 ewes in total) would have allowed a 26% prevalence to be estimated with a 95% confidence interval that has a precision (half the width of the confidence interval) of 5.9%. Enrolling 15 ewes per visit from 20 farms would allow an assumed 26% prevalence to be estimated with a 95% confidence interval that has a precision of 6.8%.

3.3.2 Study procedures

All procedures were carried out in the milking parlour by trained technicians and/or the lead author, with ewes in a standing position. Ewes were identified by visual ear tags. Body fat reserves were assessed by palpating the spinous and transverse processes of the lumbar vertebrae and assigning BCS on a five-point scale, with increments of 0.5. We used the technique described by Kenyon *et al.* (2014). When known, ewe demographic information (age, number of foetuses at pregnancy diagnosis, number of lambs born, lambing date, and first milking date) was subsequently collected from farmers by email and at an in-person interview by the lead author with each farm owner or manager after all ewes had been dried off (May-June 2023). The interview was conducted using a standardised questionnaire and the responses were recorded in a spreadsheet (Excel, Microsoft Corporation, Redmond, USA).

3.3.2.1 Assessments of teat and udder morphology

Udders were assessed for depth, suspension, gland separation, teat placement, and symmetry, using the system of Griffiths *et al*. (2019), in which the scores were scaled to a range of 1-5 (Supplementary Figure 1). Udder depth was defined as the distance between the udder cleft

(the most dorsal point of the udder skin at the junction between the glands at the apex of the udder when observed from behind the ewe) and the abdominal wall, with a score of 3 applied when the cleft was level with the hocks and a score of 5 when the cleft was against the belly. Udder suspension was defined as the ratio between the udder attachment width and the udder height, with a score of 3 indicating a ratio of 1 and a score of 5 indicating an udder with an attachment width greater than the height. Gland separation was defined as the extent to which the two glands were separated by the cleft, with a score of 1 equating to no separation (a globose udder) and a score of 5 equating to the cleft extending more than half of the udder height. Teat placement was determined from the vertical distance between the teat attachments and the most distal point of the udder, with a score of 1 indicating teats that are placed at the most dependent part of the udder and oriented downward and a score of 5 indicating teats placed high up the udder and oriented laterally. Symmetry was subjectively appraised by observing the evenness of the two gland's positions and sizes and coded as either symmetrical or asymmetrical. The presence of supernumerary teats was also noted. Teat length and width (at the attachment to the udder) were measured with Vernier callipers (Fuller 150mm Pocket Vernier Calliper, Fuller, Montreal, Canada) to the nearest millimetre (Supplementary Figure 2).

3.3.2.2 Assessments of teat and udder pathology

Inflammation, defined as any heat or redness, was recorded as present or absent for each gland and teat. External lesions, categorised as nodules, scabs, scars, or other, were recorded by type of lesion as present or absent for both teats and glands, and a description was entered for the category "other". Teat end hyperkeratosis was measured on a four-point scale developed for dairy ewes as described by Vouraki *et al.* (2018): score 1) no keratin ring around teat orifice; score 2) a smooth or slightly rough ring around the orifice and no keratin fronds; score 3) a raised roughened ring with isolated fronds of old keratin extending 1-3mm

from orifice; score 4) a raised ring with rough fronds of old keratin extending >4mm from orifice. Glands were palpated for consistency and the presence of nodules, and teats were palpated for consistency, thickening of the teat canal, and patency. Palpation and udder assessments were performed using a categorical scoring system in the manner described by Griffiths *et al.* (2019). Udder palpation was graded from 1 (soft consistency) to 7 (diffuse hard consistency), and teat palpation from 1 (soft consistency) to 5 (obstructed). The presence of weak (subjectively judged on external volume to be producing a small volume of milk compared to the contralateral gland) or dry glands ("half udders") was recorded at the ewe level.

Study personnel who performed morphology and pathology assessments for North Island farms attended a training day prior to the study commencing to calibrate their scoring, and the lead author and lead technician presided at the first three farm visits on South Island farms for calibration. The lead author oversaw visits at random across the study to re-calibrate scoring.

3.3.3 Data management

Raw physical examination data were captured using paper forms and, when mobile reception allowed, by entry directly into a custom smartphone application that stored the data in an online database. Paper forms were transcribed to spreadsheets (Excel), and online data were exported as spreadsheets (Excel).

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 categorised into hoggets (one year of age at lambing), two-tooths (two years of age at lambing), and mixed age (older than two-tooth). These variables were left blank when the data were not available.

We collected data at the gland and ewe levels. Teat morphology, and teat and gland pathology were measured at the gland level. Ewe demographic information, udder morphology, presence of supernumerary teats, and presence of half udders were measured at the ewe level. All gland-level variables were initially explored at the gland level (i.e., for the left and right sides separately) and were then, due to low prevalences, collapsed to the udder (ewe) level for analysis except for teat length and width, which were reported at the gland level. Teat end hyperkeratosis scores were categorised into three ewe-level groups per the method used by Vouraki *et al*. (2018): group 1) no or mild hyperkeratosis (ewes with both teat-ends scored <3); group 2) medium hyperkeratosis (ewes with only one teat-end scored ≥3); and group 3) severe hyperkeratosis (ewes with both teat-ends scored ≥3). If inflammation was present in at least one teat or glands, the ewe was deemed positive for teat or gland inflammation. Similarly, if any lesion was present in at least one teat or gland, the ewe was deemed positive for lesions of the teats or glands. Udder palpation scores were collapsed into ascending grades "normal", "lump", and "hard" per the methods of Griffiths *et al*. (2019), and teat palpation scores collapsed into "normal" and "abnormal" (any score >1). The ewe was assigned the worst of the two gland and teat palpation categories.

3.3.4 Statistical analysis

Unless otherwise stated, all statistical tests were two-tailed, and the critical significance level was set at 5%. Data were imported into RStudio using R 4.2.2 for statistical analysis (R Core Team 2023). The data were collated and merged in wide format by uniquely identifying each ewe and visit on each farm, then examined for completeness, duplication, consistency, and spurious values.

Exploratory data analysis included generating tables of summary statistics and distributional plots, overall and by farm, age group, and visit. Relationships between pairs of variables were

visualized with frequency tables and plots. This was performed separately for ewe-level and farm-level variables. Pairwise associations between categorical variables were tested by χ^2 analysis unless expected counts were <5, in which case Fisher's test was used with the p-value simulated from 2,000 replicates (calculation of the exact p-value was computationally demanding given the size of the dataset). Associations between categorical and continuous variables were tested by ANOVA (normally distributed continuous variables) or the Kruskal–Wallis rank-sum test (non-parametric continuous and integer variables). To test for differences in teat length and width between farms, mixed linear regression models were constructed with a fixed effect for farm and a random intercept for ewe to account for clustering within ewe. The significance of the associations between teat length and teat width and farm were tested by a likelihood ratio test against analogous models without fixed effects for farm.

An association was found between age and the prevalence of gland lesions at the ewe level during the exploratory analysis. To calculate adjusted odds ratios that accounted for clustering withing farm, a mixed generalised linear regression was built, with a random intercept for farm to account for clustering. Age was the only predictor variable. Odds ratios and their 95% confidence intervals were computed which, due to the low prevalences of gland lesions, were approximate to relative risks. The model was tested for overdispersion, goodness of fit, and homoscedasticity by inspection of simulated residual plots, and influential observations by deletion diagnostics. Linearity was not assessed because there was only a single, categorical predictor variable.

3.4 Results

Across the three visits, 893 observations were made on 882 unique ewes (11 ewes were examined at two visits by chance, though three of those were missing ear tags and were likely

six different ewes). Visits 1-3 were conducted on 24 August to 6 October 2022, 7 November to 22 December 2022, and 25 January to 16 March 2023 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 (n=3 farms) on the ewes or prolonged adverse weather (n=1 farm). The numbers of ewes examined on each farm at each visit are summarised in the supplementary materials (Supplementary Table 1). Outside of the first three visits, more than 15 ewes were examined at six farm visits due to farmer selection error and having enough time to enrol more ewes. Only 12 ewes were examined at one farm visit due to farmer error in separating the ewes from the main flock. No ewes were excluded at the selection or data analysis stages. Complete demographic and examination data were available for 326 observations. The reasons for missing data are contained in the supplementary materials (Supplementary Table 2).

3.4.1 General ewe information

Because ewes were randomly selected anew at each visit, there were differences between visits in lambing spread (the range of lambing dates) of the selected ewes, with medians of 07 August (range = 17 July - 19 September), 26 August (range = 09 July - 16 October), and 22 August (range = 02 July - 15 October) for visits 1, 2, and 3 respectively (Kruskal-Wallis p <0.001). Of the 409 ewes with data on the number of lambs born, there were 138 (33.7%), 211 (51.6%), 57 (13.9%) and 3 (0.7%) ewes with singles, twins, triplets, and quadruplets respectively. These proportions did not differ between visits (Kruskal-Wallis p = 0.2). The distributions of age at lambing, DIM at first milking, and BCS and DIM at the visit, differed between visits (Table 1).

Table 1: Median and interquartile range 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	Overall[1]	Visit 1[1]	Visit 2[1]	Visit 3[1]	p-value[2]
Age at lambing (years)	659	2 (1, 3)	3 (2, 3)	2 (1, 4)	2 (1, 3)	0.010
Lambing to first milking interval (days)	438	3 days (2 days, 41 days)	3 days (2 days, 4 days)	4 days (2 days, 63 days)	4 days (2 days, 57 days)	<0.001
Body condition score at visit	890	2.5 (2.0, 2.5)	2.5 (2.0, 2.5)	2.5 (2.0, 3.0)	2.5 (2.0, 3.0)	<0.001
Days since lambing at visit	479	102 (46, 166)	32 (26, 44)	94 (78, 118)	183 (161, 201)	<0.001

[1]Median (Q1, Q3)

[2]Kruskal-Wallis rank sum test

3.4.2 Teat and udder morphology

Raw gland-level results and sample images are provided in Supplementary Table 3 and Supplementary Figures 3–6.

3.4.2.1 Teat-level measurements

Teat length and width were measured for 1,776 and 1,773 teats respectively. Mean (SD) teat length was 27.5 (4.9) and mean (SD) width was 15.8 (2.9) mm (Figure 1). At the farm level, mean teat length and width both varied significantly between farms ($p < 0.001$), with mean length at the farm level ranging across the 20 farms from 24.9 to 34 mm and mean width ranging from 14.2 to 17 mm (Figure 1).

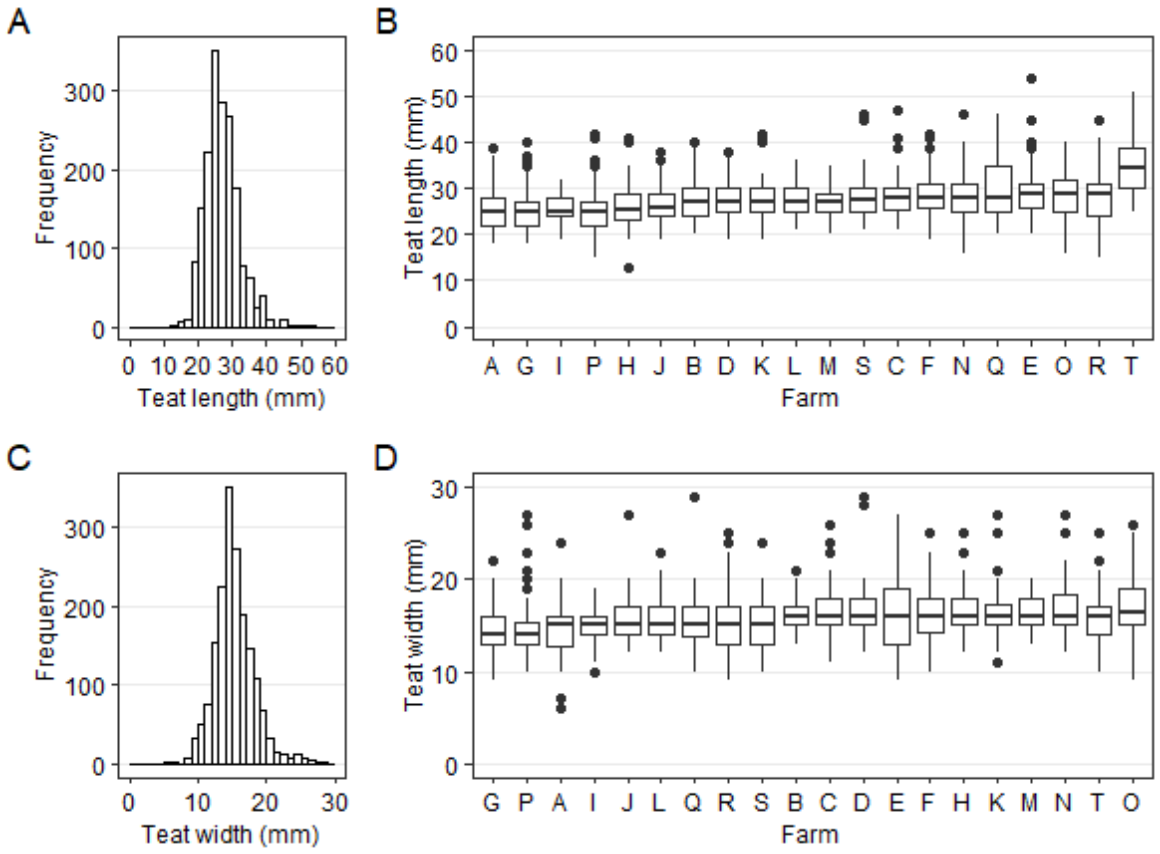

Figure 1: Distributions of teat length overall (A) and by farm (B), and teat width (at the base) overall (C) and by farm (D), in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand. Farms are ordered on their median values. Boxes extend from the 25th to the 75th percentiles, with a line at the median. Whiskers extend to values no more than 1.5 times the interquartile range, and outliers are shown as individual dots.

3.4.2.2 Udder-level measurements

Udder morphology is summarised overall and by visit in Table 2, and by age group in Table 3. Udder separation, udder suspension and teat placement scores all had modes of 3, while udder depth score had a mode of 4. The distributions of udder depth, separation, and suspension, and teat placement, were statistically different in shape between visits and age groups, but the modes remained the same across visits and age groups. The prevalence of udder asymmetry increased with age group (Table 3) but a difference between visits was not confirmed (Table 2). No difference in the prevalence of supernumerary teats was confirmed between visits or age groups. Udder depth, separation and suspension scores decreased with

age (udders were longer, less separated, and had narrower bases relative to their heights), while teat placement score and the prevalence of asymmetry increased with age (Table 3). There was between-farm variation in all udder morphology variables (Fisher exact test $p < 0.001$ for udder depth, separation, suspension, and teat placement, and presence of supernumerary teats; Fisher exact test $p = 0.021$ for udder asymmetry) (Figure 2).

Table 2: Udder morphology of ewes, by visit and overall, in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand. Denominators differ because of incomplete data collection and ewes with dry glands.

Variable	Overall	Visit 1[1]	Visit 2[2]	Visit 3[3]	p-value[4]
Udder depth					<0.001[5]
1	0/893 (0%)	0/286 (0%)	0/306 (0%)	0/301 (0%)	
2	8/893 (0.9%)	2/286 (0.7%)	4/306 (1.3%)	2/301 (0.7%)	
3	129/893 (14%)	61/286 (21%)	39/306 (13%)	29/301 (9.6%)	
4	613/893 (69%)	195/286 (68%)	219/306 (72%)	199/301 (66%)	
5	143/893 (16%)	28/286 (9.8%)	44/306 (14%)	71/301 (24%)	
Udder separation					<0.001[5]
1	26/890 (2.9%)	14/284 (4.9%)	8/305 (2.6%)	4/301 (1.3%)	
2	291/890 (33%)	115/284 (40%)	86/305 (28%)	90/301 (30%)	
3	383/890 (43%)	118/284 (42%)	133/305 (44%)	132/301 (44%)	
4	162/890 (18%)	34/284 (12%)	67/305 (22%)	61/301 (20%)	
5	28/890 (3.1%)	3/284 (1.1%)	11/305 (3.6%)	14/301 (4.7%)	
Udder suspension					<0.001[5]
1	39/893 (4.4%)	7/286 (2.4%)	8/306 (2.6%)	24/301 (8.0%)	
2	252/893 (28%)	82/286 (29%)	77/306 (25%)	93/301 (31%)	
3	346/893 (39%)	114/286 (40%)	130/306 (42%)	102/301 (34%)	
4	182/893 (20%)	68/286 (24%)	71/306 (23%)	43/301 (14%)	
5	74/893 (8.3%)	15/286 (5.2%)	20/306 (6.5%)	39/301 (13%)	
Teat placement					0.004[5]
1	0/893 (0%)	0/286 (0%)	0/306 (0%)	0/301 (0%)	
2	76/893 (8.5%)	25/286 (8.7%)	21/306 (6.9%)	30/301 (10.0%)	
3	450/893 (50%)	122/286 (43%)	174/306 (57%)	154/301 (51%)	
4	304/893 (34%)	124/286 (43%)	90/306 (29%)	90/301 (30%)	
5	63/893 (7.1%)	15/286 (5.2%)	21/306 (6.9%)	27/301 (9.0%)	
Udder asymmetry	345/891 (39%)	104/285 (36%)	132/306 (43%)	109/300 (36%)	0.15[6]
Supernumerary teat(s)	135/884 (15%)	32/280 (11%)	53/304 (17%)	50/300 (17%)	0.093[6]

[1]Aug-Oct 2022.

[2]Nov-Dec 2022.

[3]Jan-Mar 2023.

[4]Fisher's exact test or Pearson's Chi-squared test for differences in the distributions of each variable's scores (1-5) across visits.

[5]Fisher's Exact Test for Count Data with simulated p-value (based on 2000 replicates)

[6]Pearson's Chi-squared test

Table 3: Udder morphology of ewes, by age group and overall, in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand. Denominators differ because of incomplete data collection and ewes with dry glands.

Variable	Overall	Hogget[1]	2-tooth[2]	MA[3]	p-value[4]
Udder depth					<0.001[5]
1	0/893 (0%)	0/168 (0%)	0/171 (0%)	0/554 (0%)	
2	8/893 (0.9%)	1/168 (0.6%)	0/171 (0%)	7/554 (1.3%)	
3	129/893 (14%)	7/168 (4.2%)	27/171 (16%)	95/554 (17%)	
4	613/893 (69%)	97/168 (58%)	118/171 (69%)	398/554 (72%)	
5	143/893 (16%)	63/168 (38%)	26/171 (15%)	54/554 (9.7%)	
Udder separation					0.019[5]
1	26/890 (2.9%)	1/168 (0.6%)	5/171 (2.9%)	20/551 (3.6%)	
2	291/890 (33%)	38/168 (23%)	60/171 (35%)	193/551 (35%)	
3	383/890 (43%)	81/168 (48%)	75/171 (44%)	227/551 (41%)	
4	162/890 (18%)	41/168 (24%)	27/171 (16%)	94/551 (17%)	
5	28/890 (3.1%)	7/168 (4.2%)	4/171 (2.3%)	17/551 (3.1%)	
Udder suspension					<0.001[5]
1	39/893 (4.4%)	0/168 (0%)	8/171 (4.7%)	31/554 (5.6%)	
2	252/893 (28%)	20/168 (12%)	51/171 (30%)	181/554 (33%)	
3	346/893 (39%)	65/168 (39%)	62/171 (36%)	219/554 (40%)	
4	182/893 (20%)	43/168 (26%)	42/171 (25%)	97/554 (18%)	
5	74/893 (8.3%)	40/168 (24%)	8/171 (4.7%)	26/554 (4.7%)	
Teat placement					0.026[5]
1	0/893 (0%)	0/168 (0%)	0/171 (0%)	0/554 (0%)	
2	76/893 (8.5%)	23/168 (14%)	9/171 (5.3%)	44/554 (7.9%)	
3	450/893 (50%)	92/168 (55%)	81/171 (47%)	277/554 (50%)	
4	304/893 (34%)	46/168 (27%)	65/171 (38%)	193/554 (35%)	
5	63/893 (7.1%)	7/168 (4.2%)	16/171 (9.4%)	40/554 (7.2%)	
Udder asymmetry	345/891 (39%)	45/168 (27%)	64/170 (38%)	236/553 (43%)	0.001[6]
Supernumerary teat(s)	135/884 (15%)	24/166 (14%)	27/169 (16%)	84/549 (15%)	>0.9[6]

[1]1 year old at lambing.

[2]2 years old at lambing.

[3]Mixed age (>2 years old at lambing).

[4]Fisher's exact test or Pearson's Chi-squared test for differences in the distributions of each variable's scores (1-5) across age groups.

[5]Fisher's Exact Test for Count Data with simulated p-value (based on 2000 replicates)

[6]Pearson's Chi-squared test

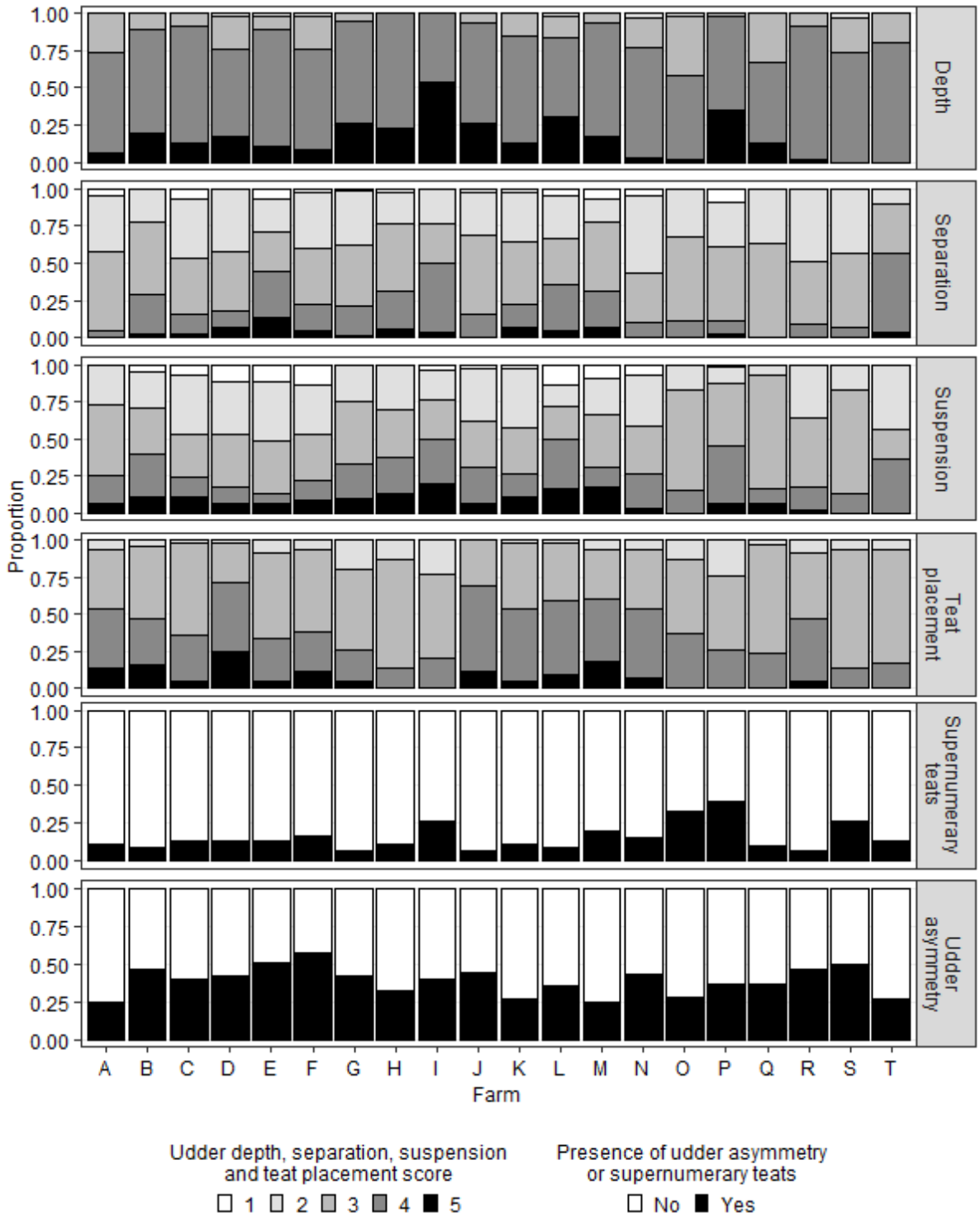

Figure 2: Distributions of udder morphology measures on each farm in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand.

3.4.3 Udder and teat pathology

Raw gland-level results and sample images are provided in the supplementary materials (Supplementary Table 3).

Overall, the ewe-level prevalence of teat end hyperkeratosis, inflammation of the glands or teats, lesions of the glands or teats, palpable defects of the glands or teats, half udders were 6% or lower (Table 4). The most common gland lesion type at the gland (ewe) level was “scab” (n=44 glands), followed by “other” (n=37 glands), nodules (n=16 glands), and scars (n=1 gland). Among the “other” category, pustules (n=17 glands), skin tags (n=4 glands), and loose skin (n=2 glands), were the most common lesions. The most common teat lesion type was “other”, observed in 15 teats, followed by “scab” (n=8 teats), nodules (n=3 teats), and scars (n=2 teats). Among the “other” category, pustules (n=4 teats) and skin tags (n=4 teats) were the most common lesions.

Table 4: Prevalence of udder and teat pathology, by visit and overall, in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand. Denominators differ because of incomplete data collection and ewes with dry glands.

Variable[1]	Overall	Visit 1[2]	Visit 2[3]	Visit 3[4]	p-value[5]
Teat end hyperkeratosis score					0.4
Group 1	874/883 (99%)	281/282 (100%)	298/301 (99%)	295/300 (98%)	
Group 2	6/883 (0.7%)	1/282 (0.4%)	1/301 (0.3%)	4/300 (1.3%)	
Group 3	3/883 (0.3%)	0/282 (0%)	2/301 (0.7%)	1/300 (0.3%)	
Inflammation - glands	15/888 (1.7%)	12/283 (4.2%)	1/305 (0.3%)	2/300 (0.7%)	<0.001
Inflammation - teats	4/889 (0.4%)	0/284 (0%)	4/305 (1.3%)	0/300 (0%)	0.042
Lesions - glands	35/883 (4.0%)	13/284 (4.6%)	3/305 (1.0%)	19/294 (6.5%)	<0.001
Lesions - teats	19/890 (2.1%)	3/284 (1.1%)	7/305 (2.3%)	9/301 (3.0%)	0.3
Palpation - glands					0.013
Normal	871/893 (98%)	273/286 (95%)	303/306 (99%)	295/301 (98%)	
Lump	22/893 (2.5%)	13/286 (4.5%)	3/306 (1.0%)	6/301 (2.0%)	
Hard	0/893 (0%)	0/286 (0%)	0/306 (0%)	0/301 (0%)	
Palpation - teats					0.009
Normal	839/893 (94%)	275/286 (96%)	277/306 (91%)	287/301 (95%)	
Abnormal	54/893 (6.0%)	11/286 (3.8%)	29/306 (9.5%)	14/301 (4.7%)	
Half udder	11/893 (1.2%)	5/286 (1.7%)	1/306 (0.3%)	5/301 (1.7%)	0.2

[1]Variables measured at the gland level (all variables except half udder) are presented at the ewe level. Teat end hyperkeratosis was first scored at the gland level using the method of Vouraki *et al*. (2018) as score 1 (no keratin ring around teat orifice); score 2 (a smooth or slightly rough ring around the orifice and no keratin fronds); score 3 (a raised roughened ring with isolated fronds of old keratin extending 1-3mm from orifice); and score 4 (a raised ring with rough fronds of old keratin extending >4mm from orifice), and was then classified at the ewe level into: group 1) no or mild hyperkeratosis; group 2) medium hyperkeratosis (ewes with only one teat-end scored ≥3); and group 3) severe hyperkeratosis (ewes with both teat-ends scored ≥3). Ewes were deemed positive for inflammation and/or lesions of the teats or glands if inflammation or lesions were recorded in either or both glands/teats. Ewes were assigned the worst of the teat and gland palpation scores. A half udder was subjectively defined as producing a small volume of milk compared to the contralateral gland, or to be non-lactating.

[2]Aug-Oct 2022.

[3]Nov-Dec 2022.

[4]Jan-Mar 2023.

[5]Fisher's exact test.

The ewe-level prevalence of inflammation of the glands or teats, gland lesions, and palpable defects of the glands or teats differed between visits (Table 4). Inflammation and palpable lumps of the gland were more prevalent at visit 1, while visible gland lesions were more prevalent at visits 1 and 3. Palpable teat abnormalities were more prevalent at visit 2. Aside from the prevalence of lesions of the glands, which increased with age, no differences were

confirmed in the prevalence of any of the teat or udder pathology measures between age groups (Table 5). Compared to a hogget, the odds of gland lesions, after adjusting for clustering within farm, were 5.5 (95% CI = 0.6-47.8) times higher for two-tooth ewes, and 10.8 (95% CI = 1.4-81.8) times higher for mixed-age ewes.

Table 5: Prevalence of udder and teat pathology, by age group and overall, in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand. Denominators differ because of incomplete data collection and ewes with dry glands.

Variable[1]	Overall	Hogget[2]	2-tooth[3]	MA[4]	p-value[5]
Teat end hyperkeratosis score					0.11
Group 1	874/883 (99%)	165/167 (99%)	168/171 (98%)	541/545 (99%)	
Group 2	6/883 (0.7%)	2/167 (1.2%)	3/171 (1.8%)	1/545 (0.2%)	
Group 3	3/883 (0.3%)	0/167 (0%)	0/171 (0%)	3/545 (0.6%)	
Inflammation - glands	15/888 (1.7%)	1/166 (0.6%)	3/171 (1.8%)	11/551 (2.0%)	0.6
Inflammation - teats	4/889 (0.4%)	1/167 (0.6%)	0/171 (0%)	3/551 (0.5%)	0.8
Lesions - glands	35/883 (4.0%)	1/168 (0.6%)	5/170 (2.9%)	29/545 (5.3%)	0.012
Lesions - teats	19/890 (2.1%)	1/168 (0.6%)	3/171 (1.8%)	15/551 (2.7%)	0.2
Palpation - glands					0.2
Normal	871/893 (98%)	167/168 (99%)	166/171 (97%)	538/554 (97%)	
Lump	22/893 (2.5%)	1/168 (0.6%)	5/171 (2.9%)	16/554 (2.9%)	
Hard	0/893 (0%)	0/168 (0%)	0/171 (0%)	0/554 (0%)	
Palpation - teats					0.8
Normal	839/893 (94%)	159/168 (95%)	162/171 (95%)	518/554 (94%)	
Abnormal	54/893 (6.0%)	9/168 (5.4%)	9/171 (5.3%)	36/554 (6.5%)	
Half udder	11/893 (1.2%)	0/168 (0%)	3/171 (1.8%)	8/554 (1.4%)	0.2

[1]Variables measured at the gland level (all variables except half udder) are presented at the ewe level. Teat end hyperkeratosis was first scored at the gland level using the method of Vouraki *et al*. (2018) as score 1 (no keratin ring around teat orifice); score 2 (a smooth or slightly rough ring around the orifice and no keratin fronds); score 3 (a raised roughened ring with isolated fronds of old keratin extending 1-3mm from orifice); and score 4 (a raised ring with rough fronds of old keratin extending >4mm from orifice), and was then classified at the ewe level into: group 1) no or mild hyperkeratosis; group 2) medium hyperkeratosis (ewes with only one teat-end scored ≥3); and group 3) severe hyperkeratosis (ewes with both teat-ends scored ≥3). Ewes were deemed positive for inflammation and/or lesions of the teats or glands if inflammation or lesions were recorded in either or both glands/teats. Ewes were assigned the worst of the teat and gland palpation scores. A half udder was subjectively defined as producing a small volume of milk compared to the contralateral gland, or to be non-lactating.

[2]1 year old at lambing.

[3]2 years old at lambing.

[4]Mixed age (>2 years old at lambing).

[5]Fisher's exact test

There was between-farm variation in the prevalence of teat lesions (Fisher exact test p = 0.014) and teat palpation scores (Fisher exact test p = 0.002), but such differences were not confirmed for teat end hyperkeratosis, inflammation of the glands or teats, lesions of the glands, palpation of the glands, or the prevalence of half udders (Fisher exact test p = 0.2, 0.3, 0.5, 0.12, 0.7, and 0.2 respectively) (Figure 3). Group 2 hyperkeratosis was only observed on four farms, with prevalences of 2.2-5.0%, and group 3 hyperkeratosis on three farms, with prevalences of 1.7-2.3%.

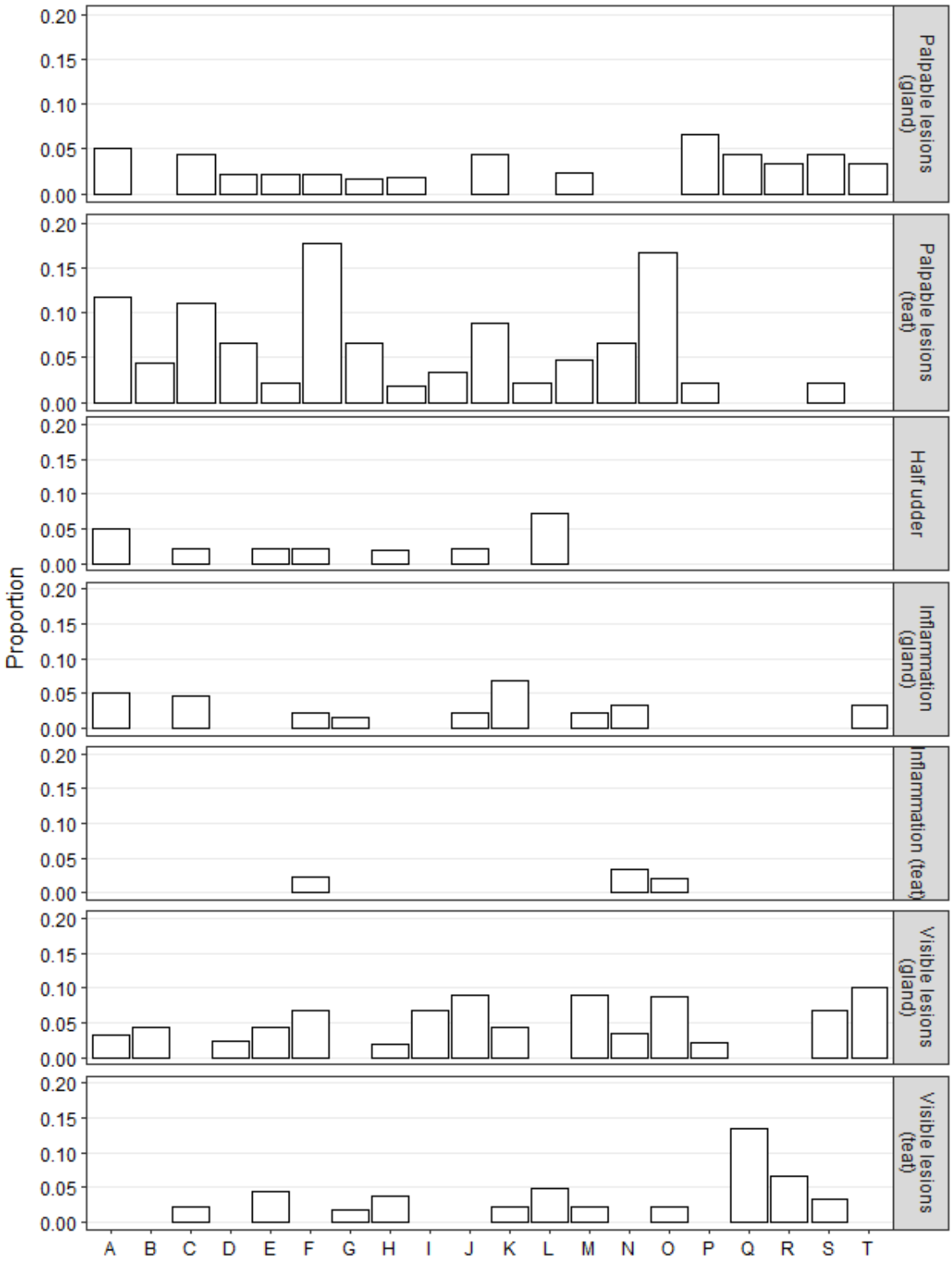

Figure 3: Prevalence of teat and udder pathology measures on each farm in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand. Missing bars indicate a prevalence of zero.

3.5 Discussion

We present here the first large scale study of teat and udder morphology and pathology from dairy ewes on multiple farms in New Zealand. The purpose was to establish a baseline set of data that represents the New Zealand situation at this stage in the industry's development. With that baseline, the variability between flocks can be understood, farmers and industry partners can compare their own flocks to the results in this dataset, areas for improvement can be identified, and future studies can be compared to this one. Approximately 50% of commercial flocks were enrolled, providing a strong representation of the industry at this time.

3.5.1 Teat and udder morphology

There are limited published studies of teat and udder morphology in dairy ewes, and direct comparisons are generally not possible because different scales have been used. A study of primiparous Sardinian breed ewes found the same preponderance of mid-range scores (on a 1-9 scale) for suspension, (mean = 5.11), but higher udder depth (less pendulous udders), separation, and teat placement scores (means = 6.38, 6.46, and 7.32 respectively) (Casu *et al.* 2006). These differences may however be due to parity. On the other hand, the ewes in the present study had higher udder depth but similar separation, suspension, and teat placement scores to those observed among East Friesian-cross ewes in the Czech Republic, who recorded means of 3.82, 5.12, 4.81, and 4.06 for depth, separation, suspension, and teat placement respectively using a nine-point scale (Makovický *et al.* 2024). Among Churra breed ewes in Spain, the udder depth, attachment, and teat placement scores resembled those in the present study, with means of 5.16 (with high scores corresponding to more pendulous udders), 5.14, and 4.48 respectively (Fuente *et al.* 1996). Differences between New Zealand dairy ewes and dairy ewes in other countries may be partly due to the industry being relatively young in New Zealand, with few farms or breeders having had many decades to

select ewes for milking and milk production. Griffiths *et al*. (2019) used the same scale as the present study for non-dairy New Zealand ewes and found similar scores for suspension (mode = 3-5 depending on the visit), and teat placement (mode = 3), results but higher udder depth scores (mode = 3-5 depending on the visit) and lower separation scores (mode = 1). The lower depth scores in the present study were likely a result of the greater udder volume in dairy ewes compared to non-dairy ewes. The teat dimensions observed in the present study were like those observed in eight European dairy breeds by Labussiere (1988), who reported means lengths of 26.1-33.3 mm and mean widths of 14.3-17.9 mm, but shorter than those of East Friesian-cross ewes in the Czech Republic (Makovický *et al.* 2024), who reported mean lengths of 37.0 mm.

We found differences in udder morphology between visits, though the differences were relatively small, and the modes of each udder morphology measure remained the same throughout the season. We did not sample the same ewes at each visit, so some of the differences are likely to be due to the different ewes sampled at each visit rather than a true change in the population. However, stage of lactation was shown to affect udder depth, width, and circumference, cistern height, and teat position, angle, length, and width by Fernández *et al*. (1995). This may be due to changes in milk volume affecting udder fullness and therefore morphology.

Among older ewes, udders were longer, less separated, had narrower bases relative to their heights, and the teats were more laterally placed. Other researchers have noted changes with parity. Sezenler *et al*. (2016) found udder circumference and width increased with parity, while teat length and diameter were not affected. In a study of Spanish breeds, udder depth increased with parity while suspension, teat angle, and udder shape scores declined, and teat length remained the same (Rovai *et al.* 2004).

Substantial differences in morphology were found between farms. There are no other published literature comparing morphology across farms to our knowledge. Breed, age, and lactation stage may contribute some of the differences between farms, but this descriptive study was not designed to explain such differences. We did not examine associations between breed and morphology due to the incompleteness of breed information and the large number of breeds and cross breeds on the study farms. In other studies, breed effects on udder length, distance between teats and teat angle were non-significant (Caja *et al.* 2000).

Udder symmetry was subjectively assessed, considering the relative size and position of each gland. Only a single published study that reported symmetry as a binary measurement was found (Griffiths *et al.* 2019). The study enrolled New Zealand non-dairy ewes and sampled them at pre-mating (February), pre-lambing (October), docking (November) and weaning (January 2018), and found 5-10% of the (non-dairy) ewes having asymmetric udders depending on the time point. Asymmetry was much more prevalent in the present study, and we noted an increase in asymmetry as parity increased, suggesting an accumulation of damage or pathology. However, the prevalence did not increase across the lactation, though it is possible that farmers removed ewes with more severe asymmetry from the milking flock.

Approximately one in seven ewes had supernumerary teats, with no differences between parities. While they are common and heritable, Spanish research suggests that they do not present a significant problem and need not be removed (Palacios and Abecia 2014).

It is not clear what the ideal udder conformation is for a New Zealand dairy ewe, and it probably depends on the farm system. Farmers who rear lambs on the ewe may require different morphology characteristics, especially teat placement and udder depth (higher teat placement and less pendulous udders being more favourable for suckling lambs) than farmers who remove lambs within a week of lambing. Udders that are more pendulous (and therefore

higher in volume), lower suspension scores (narrower attachments), greater separation scores, and lower teat placement scores, are optimal for production and milkability (Sagi and Morag 1974; Marshall *et al.* 2023), though excessively pendulous udders can be harder to milk. But there are trade offs between milkability and mastitis, and deeper udders are more prone to physical injury. For example, lower udder depth scores (more pendulous udders) are associated with higher somatic cell counts (Marshall *et al.* 2023), which are a proxy for mastitis. However, this antagonistic relationship appears to be mitigated by selecting for somatic cell count (Allain *et al.* 2018). In general, moderately low udder depth scores (moderately pendulous) and teat placement scores, and moderately high suspension and separation scores, are likely to be ideal for farmers who machine milk ewes and rear lambs artificially. The udder depth, separation, and suspension scores in the present study were all in the mid-range, showing significant potential for improvement of the New Zealand dairy sheep flock by applying selection pressure on these traits across the industry.

3.5.2 Teat and udder pathology

We found that the prevalence of teat and udder pathology was low (below 6%), such that the variables had to be collapsed to fewer categories.

There is limited information on the prevalence of teat end hyperkeratosis in dairy ewes. Vouraki *et al*. (2018) found group 2 (mild) teat end hyperkeratosis in 8.5% and 3 (severe) teat end hyperkeratosis in 8.9% of the 1,360 ewes they examined on 60 Greek dairy sheep farms. In contrast, the prevalence of hyperkeratosis in the present study was substantially lower, with group 2 teat end hyperkeratosis found in 0.7% and group 3 teat end hyperkeratosis found in 0.3% of ewes. The prevalence did not change across visits or parities. It is not clear why the prevalence of severe hyperkeratosis was much lower in the present study, but it may

reflect shorter milking times (due to lower volume), different vacuum and pulsation settings, different teat cup liners, or different teat sprays (Vouraki *et al.* 2018).

Inflammation of the skin was also rare, but more prevalent for udders at the first visit and for teats at the second visit. The reasons for these differences across time are unknown but may reflect increased blood flow during early lactation (udders), inflammation associated with parturition (udders), or exposure to milking machinery (teats).

Lesions were similarly rare and dominated by warts, skin tags, loose skin, pustules, and scabs. It is not clear why there was a lower prevalence of gland lesions at the second visit (1.0%) than at the first (4.6) or third visit (6.5%). The increasing prevalence with parity may reflect changes in udder morphology that make the udder more prone to injury.

Minimal published information on the prevalence of palpable teat or udder defects can be found for dairy ewes. Palpable udder defects (hardness or lumps) were found in 5.0-7.4% of ewes, and palpable teat abnormalities (in one or both teats) in 6.4-34.9% of ewes across four visits in a New Zealand study of non-dairy ewes (Griffiths *et al.* 2019). The prevalences were substantially lower in the present study. The higher prevalence among non-dairy ewes may be a function of damage caused by lamb suckling. On the other hand, regular observation and culling by dairy farmers may reduce the prevalence among dairy ewes. Along with inflammation, palpable teat abnormalities were more common at the second visit and udder abnormalities at the first visit. The reasons for these differences are unknown but may reflect the same factors as for inflammation of the skin.

Between-farm differences in udder and teat pathology were only confirmed for teat lesion prevalences and udder palpation scores. Teat lesions were only found on 11/20 farms, and on those farms the prevalence varied from 1.7-13.0%. Similarly, differences in udder palpation scores were small, with prevalences of palpable lesions of 0-7% across the farms. Overall,

teat and udder pathology did not vary substantially between farms, suggesting broad similarity across the study farms, although the low prevalence of most lesions and the inclusion of only 20 farms limited the power to detect farm-level differences.

Large scale studies on commercial farms have limitations. To determine if morphology and pathology changed across the lactation, the cross sections took place at early, mid, and late lactation, in line with the work of Fthenakis (1994). Ideally, we would have sampled the same ewes at each visit, but this would have necessitated a larger sample size to address loss to follow up and required more work by the farmers to select the study ewes. The timing of udder and teat examination relative to milking was not fully standardised across farms. Most examinations were conducted two to three hours after morning milking, but ewes on four farms were examined before morning milking for at least one visit. This may have affected observations of transient features, such as udder fill, teat congestion or oedema, but was less likely to affect more persistent traits such as udder conformation, supernumerary teats, established teat-end hyperkeratosis or marked udder asymmetry. Missing demographic data limited our ability to make some comparisons across age groups and prevented comparisons by other factors such as breed. Many farmers do not routinely record such information at the individual ewe level, as ewes are often managed in mobs. The missing data were not due to the study design, but rather to the practicalities of working on commercial farms. Being commercial ventures, the prevalence of pathology at each visit may have been biased by culling.

Conclusions: A baseline set of descriptive teat and udder morphology and pathology data have been provided from 20 commercial New Zealand dairy sheep farms. Morphology was like that observed in overseas dairy sheep but varied across the season and between farms. Teat and udder pathology was rare and consistent across farms, with less pathology than has been observed in New Zealand non-dairy ewes. There is potential to improve milkability

across the industry by selection on udder morphology traits, though attention must also be paid to mastitis susceptibility. The data will be useful for farmers and industry partners to compare their own flocks to the results in this dataset, identify areas for improvement, inform future studies, and compare results from future studies against.

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3.8 Supplementary information

Supplementary Table 1. Number of ewes examined on each farm at each visit in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand.

Farm	Visit 1	Visit 2	Visit 3	Overall
A	30	15	15	60
B	15	15	15	45
C	15	15	15	45
D	15	15	15	45
E	15	15	15	45
F	15	15	15	45
G	30	15	16	61
H	15	20	18	53
I	0	15	15	30
J	15	15	15	45
K	15	15	15	45
L	15	15	12	42
M	15	15	15	45
N	30	15	15	60
O	15	16	15	46
P	16	15	15	46
Q	0	15	15	30
R	15	15	15	45
S	0	15	15	30
T	0	15	15	30
Total	286	306	301	893

Supplementary Table 2. Number of observations missing raw data, and the reasons, in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand.

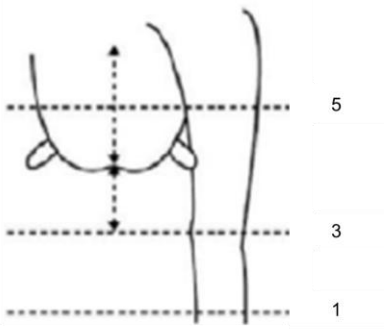
Variable	N missing	Reason
Ewe information		
Age	234	Unknown by farmer (n=234)
Body condition score	3	Not recorded (n=3)
Breed	302	Unknown by farmer (n=302)
Recorded no. lambs born	484	Unknown by farmer (n=484)
Recorded lambing date	414	Unknown by farmer (n=414)
Recorded first milking date	454	Unknown by farmer (n=454)
Udder and teat morphology		
Udder depth	0	
Udder separation	3	Pronounced asymmetry (n=3)
Udder suspension	0	
Teat placement	0	
Supernumerary teats	9	Not recorded (n=9)
Udder symmetry	2	Not recorded (n=2)
Teat length - left	4	Not recorded (n=4)
Teat length - right	6	Not recorded (n=4), half udder (n=1), spurious value (n=1)
Teat width - left	5	Not recorded (n=4, spurious value (n=1))
Udder and teat pathology		
Teat width - right	8	Not recorded (n=6), half udder (n=2)
Teat end hyperkeratosis - left	8	Not recorded (n=8)
Teat end hyperkeratosis - right	10	Not recorded (n=10)
Inflammation - left gland	4	Not recorded (n=4)
Inflammation - right gland	4	Not recorded (n=4)
Inflammation - left teat	3	Not recorded (n=3)
Inflammation - right teat	3	Not recorded (n=3)
Lesions - left gland	7	Not recorded (n=7)
Lesions - right gland	6	Not recorded (n=6)
Lesions - left teat	6	Not recorded (n=6)
Lesions - right teat	7	Not recorded (n=7)
Palpation - left gland	2	Not recorded (n=2)
Palpation - right gland	2	Not recorded (n=2)
Palpation - left teat	3	Not recorded (n=3)
Palpation - right teat	2	Not recorded (n=2)

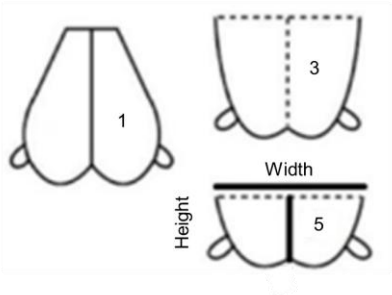
Supplementary Table 3. Gland-level prevalence of udder and teat pathology in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand.

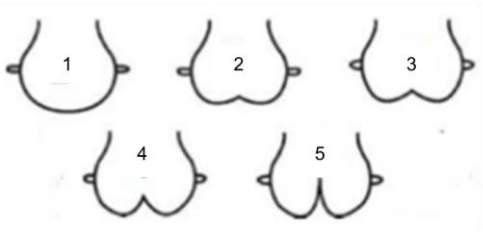
Variable	Visit 1	Visit 2	Visit 3	Overall
Inflammation - left gland	11/284 (3.9%)	0/305 (0%)	2/300 (0.7%)	13/889 (1.5%)
Inflammation - right gland	6/283 (2.1%)	1/305 (0.3%)	2/301 (0.7%)	9/889 (1.0%)
Inflammation - left teat	0/285 (0%)	3/305 (1.0%)	0/300 (0%)	3/890 (0.3%)
Inflammation - right teat	0/284 (0%)	2/305 (0.7%)	0/301 (0%)	2/890 (0.2%)
Palpation - left gland				
1	244/285 (86%)	279/305 (91%)	281/301 (93%)	804/891 (90%)
2	30/285 (11%)	25/305 (8.2%)	16/301 (5.3%)	71/891 (8.0%)
3	6/285 (2.1%)	0/305 (0%)	2/301 (0.7%)	8/891 (0.9%)
4	3/285 (1.1%)	1/305 (0.3%)	2/301 (0.7%)	6/891 (0.7%)
5	0/285 (0%)	0/305 (0%)	0/301 (0%)	0/891 (0%)
6	2/285 (0.7%)	0/305 (0%)	0/301 (0%)	2/891 (0.2%)
7	0/285 (0%)	0/305 (0%)	0/301 (0%)	0/891 (0%)
Palpation - right gland				
1	256/285 (90%)	279/305 (91%)	280/301 (93%)	815/891 (91%)
2	25/285 (8.8%)	25/305 (8.2%)	16/301 (5.3%)	66/891 (7.4%)
3	4/285 (1.4%)	0/305 (0%)	2/301 (0.7%)	6/891 (0.7%)
4	0/285 (0%)	1/305 (0.3%)	3/301 (1.0%)	4/891 (0.4%)
5	0/285 (0%)	0/305 (0%)	0/301 (0%)	0/891 (0%)
6	0/285 (0%)	0/305 (0%)	0/301 (0%)	0/891 (0%)
7	0/285 (0%)	0/305 (0%)	0/301 (0%)	0/891 (0%)
Palpation - left teat				
1	280/285 (98%)	279/304 (92%)	289/301 (96%)	848/890 (95%)
2	0/285 (0%)	25/304 (8.2%)	12/301 (4.0%)	37/890 (4.2%)
3	5/285 (1.8%)	0/304 (0%)	0/301 (0%)	5/890 (0.6%)
4	0/285 (0%)	0/304 (0%)	0/301 (0%)	0/890 (0%)
5	0/285 (0%)	0/304 (0%)	0/301 (0%)	0/890 (0%)
Palpation - right teat				
1	277/285 (97%)	283/305 (93%)	287/301 (95%)	847/891 (95%)
2	3/285 (1.1%)	22/305 (7.2%)	14/301 (4.7%)	39/891 (4.4%)
3	5/285 (1.8%)	0/305 (0%)	0/301 (0%)	5/891 (0.6%)
4	0/285 (0%)	0/305 (0%)	0/301 (0%)	0/891 (0%)
5	0/285 (0%)	0/305 (0%)	0/301 (0%)	0/891 (0%)
Nodules - left gland	1/282 (0.4%)	2/304 (0.7%)	5/300 (1.7%)	8/886 (0.9%)
Nodules - right gland	1/282 (0.4%)	3/305 (1.0%)	4/300 (1.3%)	8/887 (0.9%)
Nodules - left teat	0/281 (0%)	1/305 (0.3%)	1/301 (0.3%)	2/887 (0.2%)
Nodules - right teat	0/281 (0%)	1/305 (0.3%)	0/300 (0%)	1/886 (0.1%)
Scabs - left gland	13/282 (4.6%)	2/304 (0.7%)	9/300 (3.0%)	24/886 (2.7%)
Scabs - right gland	11/282 (3.9%)	1/305 (0.3%)	8/300 (2.7%)	20/887 (2.3%)
Scabs - left teat	4/281 (1.4%)	3/305 (1.0%)	0/301 (0%)	7/887 (0.8%)
Scabs - right teat	0/282 (0%)	1/304 (0.3%)	0/300 (0%)	1/886 (0.1%)

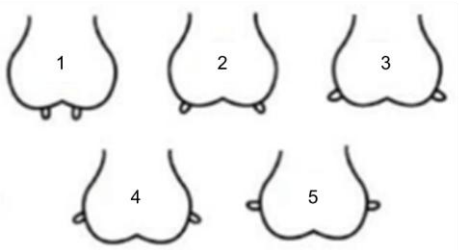
Variable	Visit 1	Visit 2	Visit 3	Overall
Scars - left gland	1/282 (0.4%)	0/304 (0%)	0/300 (0%)	1/886 (0.1%)
Scars - right gland				
0	282/282 (100%)	305/305 (100%)	300/300 (100%)	887/887 (100%)
Scars - left teat	0/281 (0%)	1/305 (0.3%)	0/301 (0%)	1/887 (0.1%)
Scars - right teat	1/282 (0.4%)	0/304 (0%)	0/300 (0%)	1/886 (0.1%)
Other - left gland	9/282 (3.2%)	4/304 (1.3%)	12/300 (4.0%)	25/886 (2.8%)
Other - right gland	5/282 (1.8%)	2/305 (0.7%)	5/300 (1.7%)	12/887 (1.4%)
Other - left teat	5/281 (1.8%)	7/305 (2.3%)	2/301 (0.7%)	14/887 (1.6%)
Other - right teat	4/282 (1.4%)	6/304 (2.0%)	1/300 (0.3%)	11/886 (1.2%)
Half udder	5/286 (1.7%)	1/306 (0.3%)	5/301 (1.7%)	11/893 (1.2%)
Teat end hyperkeratosis - left				
1	260/282 (92%)	243/303 (80%)	240/300 (80%)	743/885 (84%)
2	22/282 (7.8%)	57/303 (19%)	57/300 (19%)	136/885 (15%)
3	0/282 (0%)	2/303 (0.7%)	2/300 (0.7%)	4/885 (0.5%)
4	0/282 (0%)	1/303 (0.3%)	1/300 (0.3%)	2/885 (0.2%)
Teat end hyperkeratosis - right				
1	265/282 (94%)	241/301 (80%)	234/300 (78%)	740/883 (84%)
2	16/282 (5.7%)	58/301 (19%)	63/300 (21%)	137/883 (16%)
3	1/282 (0.4%)	1/301 (0.3%)	2/300 (0.7%)	4/883 (0.5%)
4	0/282 (0%)	1/301 (0.3%)	1/300 (0.3%)	2/883 (0.2%)

Supplementary Figure 1. Visual representation of the udder morphology scoring system developed by Griffiths *et al*. 2019 from the system of Casu *et al*. 2006, in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand.



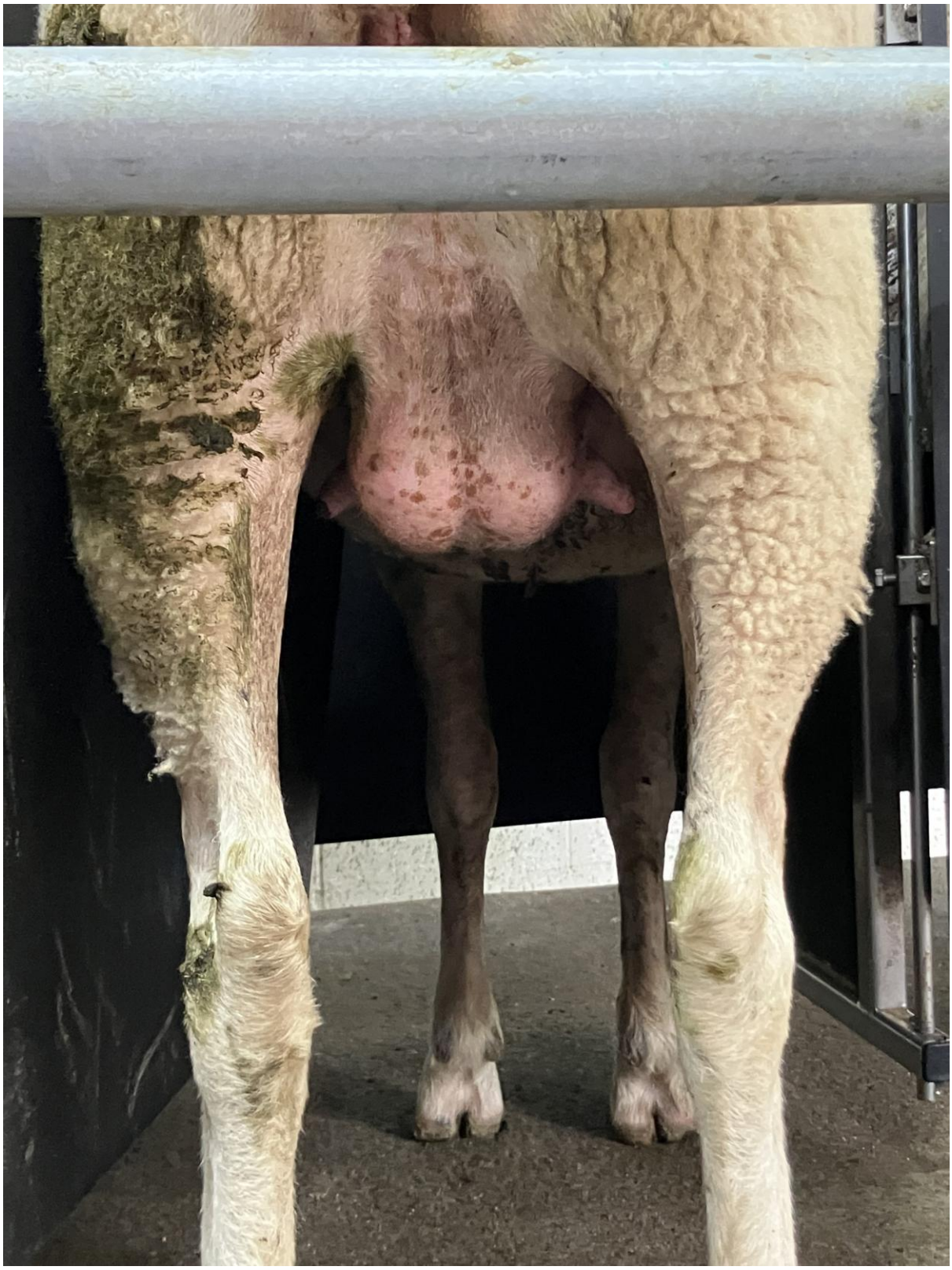




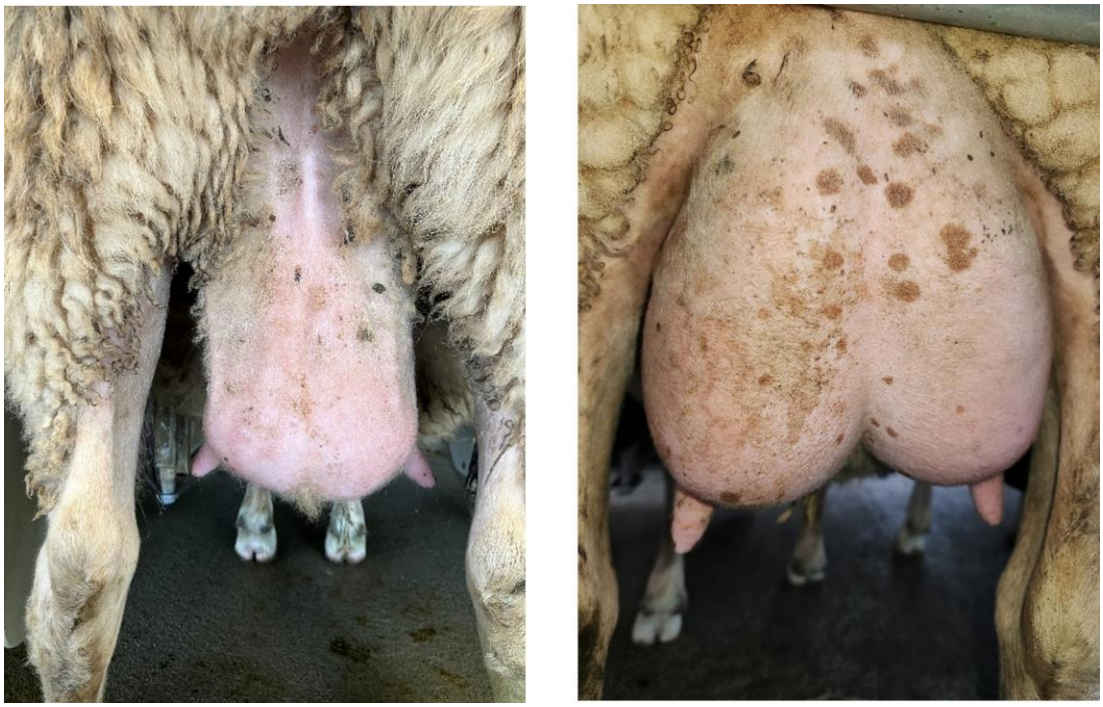


Supplementary Figure 2. Measurement of teat length and width.

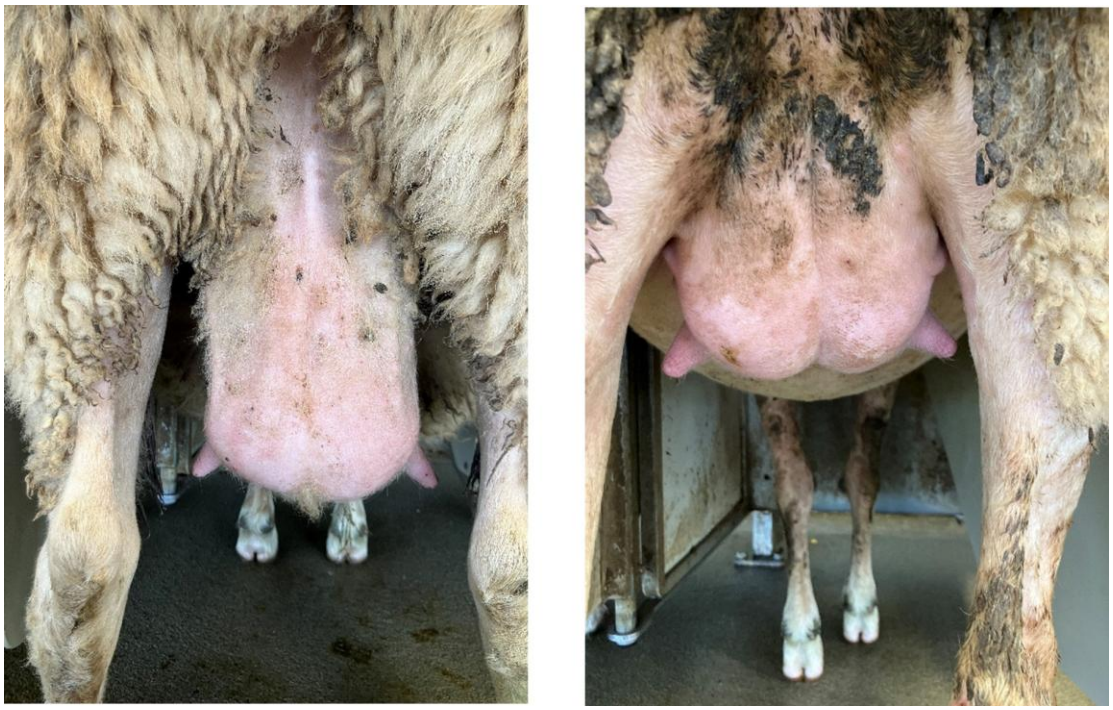
Supplementary Figure 3. Udder depth score 5.



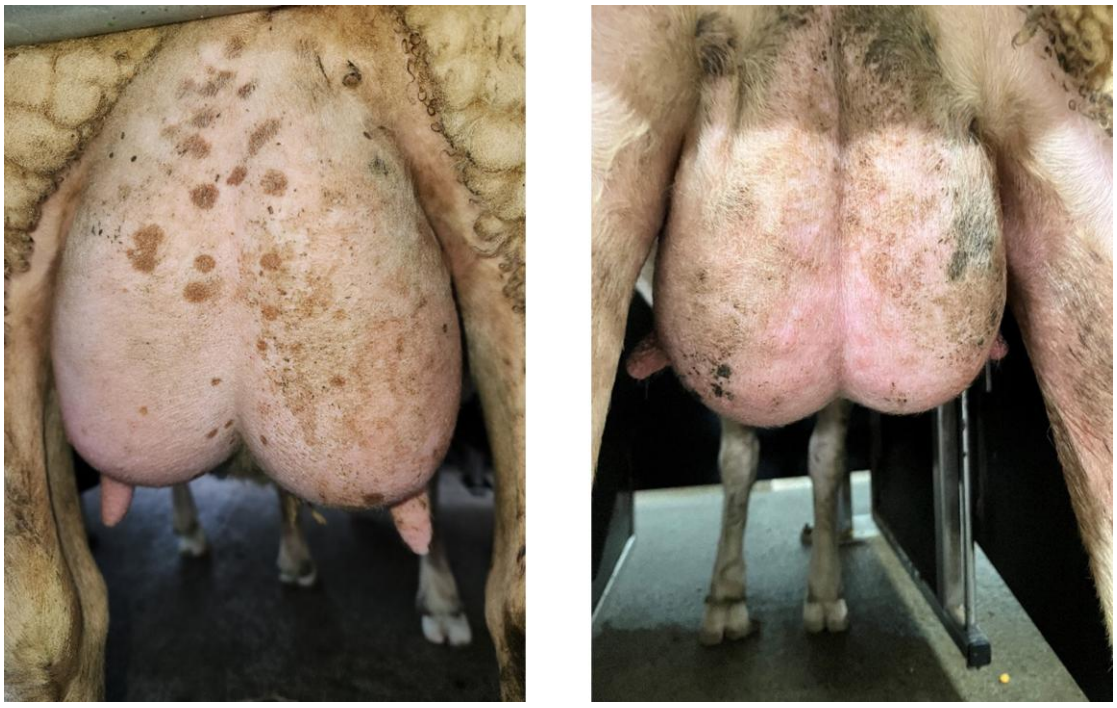
Supplementary Figure 4. Udder separation scores 1 (left) and 4 (right)



Supplementary Figure 5. Udder suspension scores 1 (left) and 5 (right).

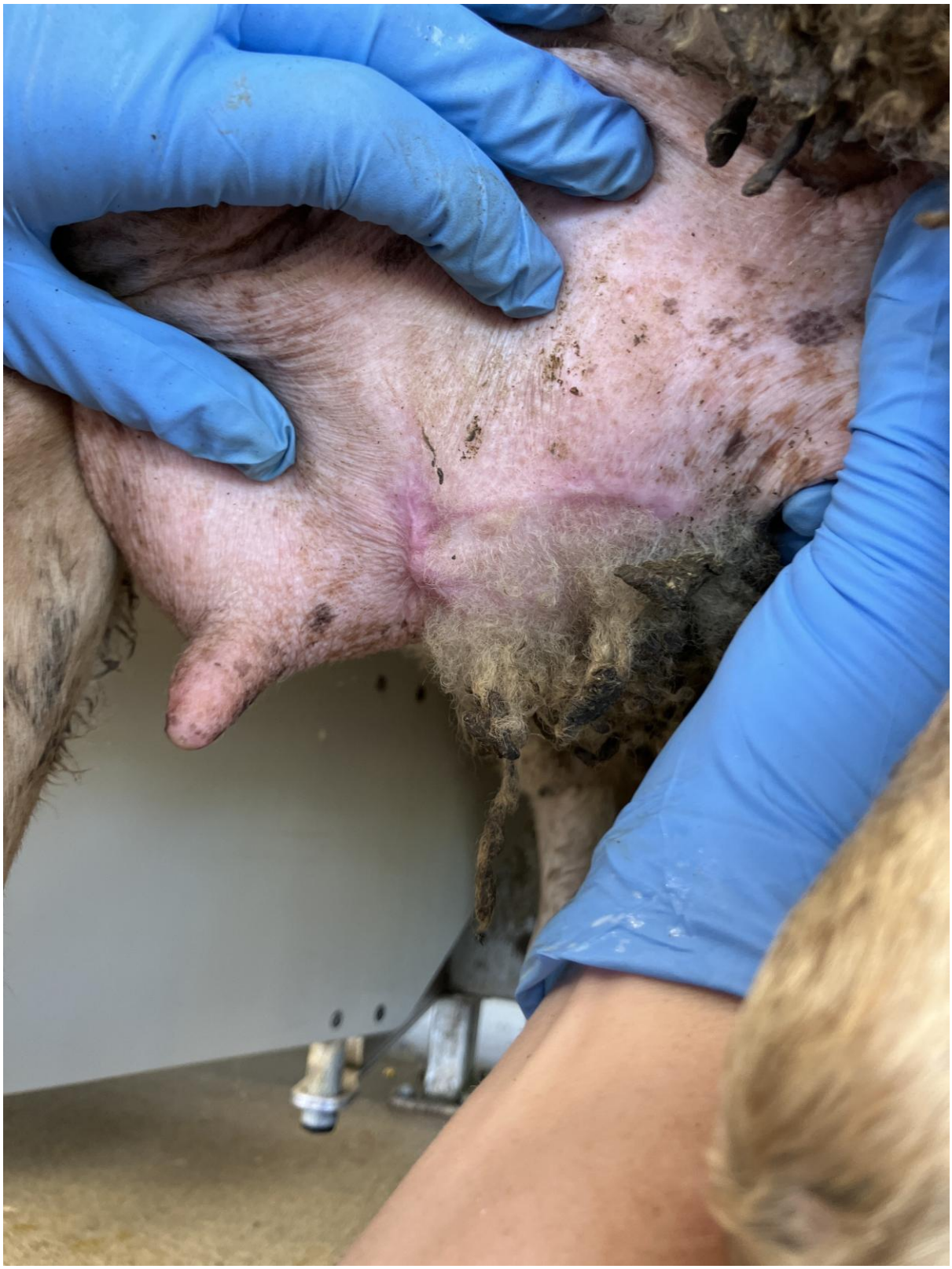


Supplementary Figure 6. Teat placement scores 2 (left) and 5 (right).

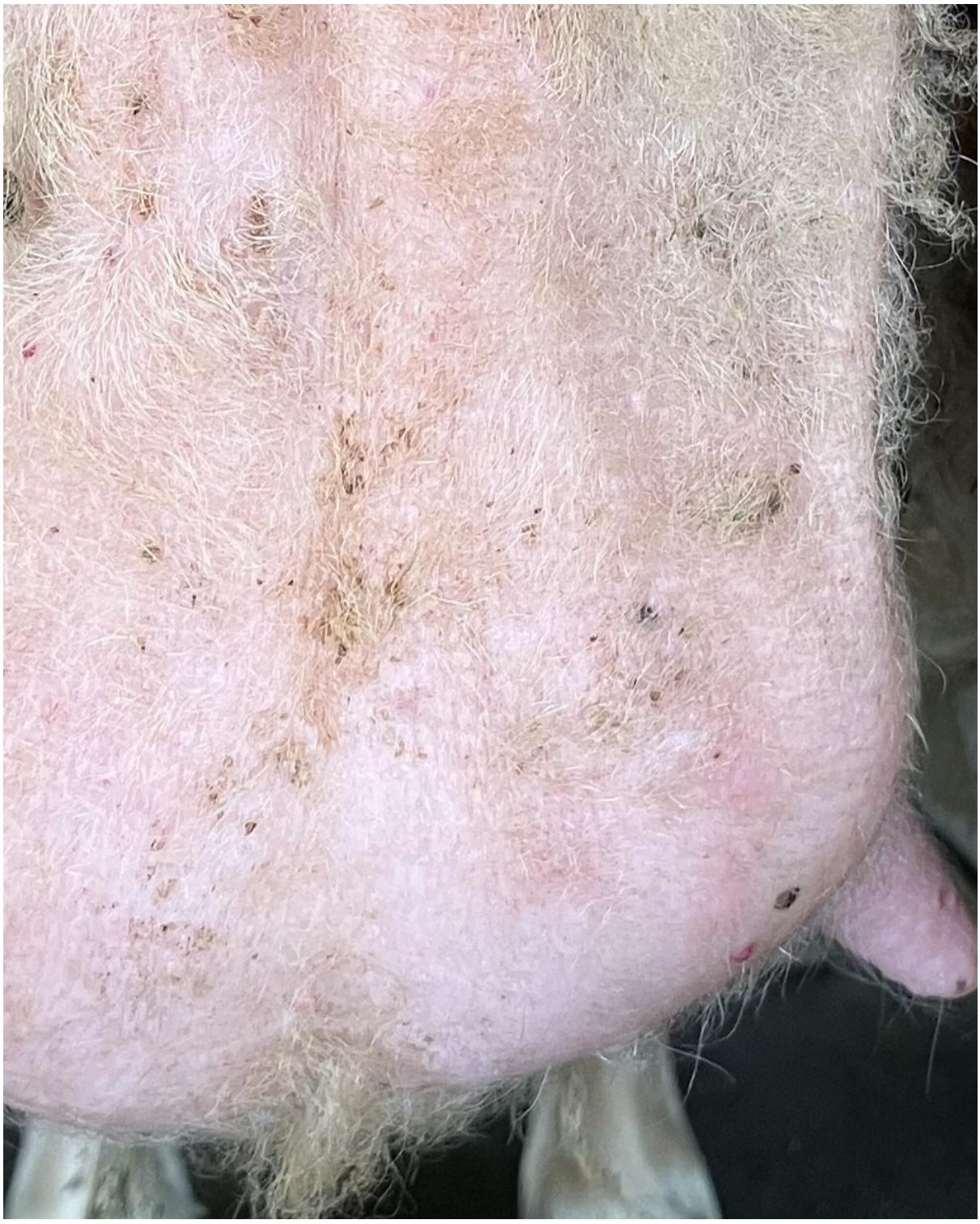


Supplementary Figure 7. Teat end hyperkeratosis score 3.

Supplementary Figure 8. Udder scar.

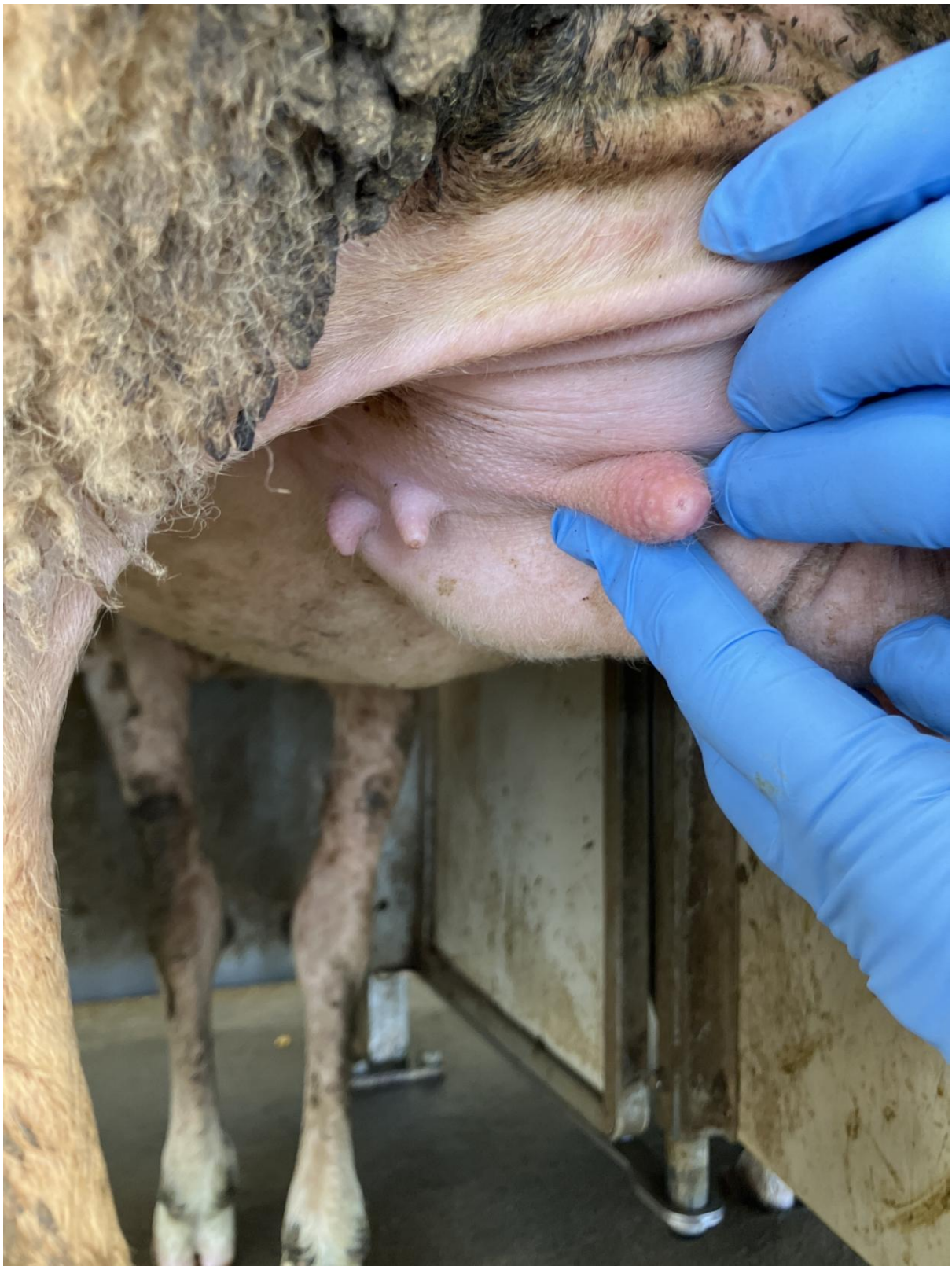


Supplementary Figure 8. Udder scabs.



Supplementary Figure 9. Udder papillomata

Supplementary Figure 10. Supernumerary teats



4 CLINICAL MASTITIS IN NEW ZEALAND DAIRY EWES

Having set the scene in the previous two chapters by characterising New Zealand dairy sheep farms and the conformation and health of ewe teats and udders, Chapter 4 is the first chapter to focus directly on mastitis.

When this research project was being designed, clinical mastitis was often the first aspect of mastitis that came to farmers' minds because it is challenging to treat, with many farmers opting to cull affected ewes immediately without attempting treatment due to poor success rates in the past. In those initial stages, it was not clear how common clinical mastitis actually was due to limitations with farm records and mastitis detection, so it became apparent that simply clarifying the incidence would be a large step forward.

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GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Greg Chambers
Name and title of main supervisor:	Professor Richard Laven
In which chapter is the manuscript/published work?	4 - Clinical mastitis

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]
The student obtained the funding, designed the protocol, collated and analysed the data, and lead the manuscript writing and responses to peer reviewers.

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Please provide the full reference of the research output:
Chambers G, Laven R, Grinberg A, Ridler A, Velathanthiri N. An observational study of farmer-reported clinical mastitis in New Zealand dairy ewes. N Z Vet J 72, 212–224, 2024

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An observational study of farmer-reported clinical mastitis in New Zealand dairy ewes.

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4.1 Abstract

Aims: To describe the incidence, aetiology, treatment, and outcomes of farmer-reported clinical mastitis on New Zealand dairy sheep farms.

Methods: A prospective cohort study was conducted on 20 spring-lambing New Zealand sheep milking farms over the 2022-2023 season. Clinical mastitis was defined as a change in the appearance of milk and/or signs of inflammation in the gland. Farmers were required to report all cases of clinical mastitis and collect information on affected ewe demographics, clinical features, treatments (where applicable), and outcomes. Milk samples from mastitic glands were submitted for microbiological culture and isolates for MALDI-TOF identification.

Results: Partial or complete clinical mastitis data were available for 236 cases from 221 ewes on 18 of the 20 study farms. Clinical mastitis was diagnosed in 0-6% of ewes at the farm level, with an overall incidence of 1.8 (95% CI =1.0-3.2) % using the study data, or 2.3 (95% CI =1.6-3.3) % using the study data and farmer estimates that included unreported cases. Cases occurred mostly in early lactation, with 59% detected during the lambing period (August to October), at a median (IQR) of 7 (3, 40) days in milk. The majority of cases featured clots in the milk (59%), swelling (55%), and unevenness (71%) of the glands.

Pyrexia (rectal temperature ≥ 40.0 °C) and depression (lethargy, inappetence, or being unable to stand) were diagnosed in 25% and 26% of cases, respectively, with overlap between clinical signs. Treatment was given to 46% of cases, with tylosin being the most commonly used treatment (50% of treated cases). The most common outcome was being instantly dried off to be culled without treatment (32%), followed by still milking and recovered with lasting problems (25%). Nearly half of all the milk samples submitted were culture negative. *Streptococcus uberis* (14%), non-aureus staphylococci (12%), and *Staphylococcus aureus* (11%) were the most common isolates, but their farm-level distribution differed: *S. uberis* was isolated from 12/16 farms that submitted milk samples for culture, whereas non-aureus staphylococci and *S. aureus* were each isolated from 8/16 farms.

Conclusions: Clinical mastitis affected up to 6% of ewes at the farm level. Systemic signs were observed in one quarter of affected ewes, suggesting a role for supportive treatment. Clinical mastitis can be severe and challenging to fully resolve in New Zealand dairy sheep.

Clinical relevance: This is the first systematic study of clinical mastitis in New Zealand dairy ewes. It provides baseline information that is specific to New Zealand conditions for farmers, veterinarians and other advisors to guide the management of mastitis for the relatively new dairy sheep industry in New Zealand.

Keywords: Clinical mastitis, ovine, incidence, microbiology, clinical features, outcomes.

Abbreviations: MALDI-TOF: matrix assisted laser desorption ionisation time-of-flight mass spectrometry; NAS: non-aureus staphylococci.

4.2 Introduction

Sheep milking is a small but growing industry in New Zealand that has not yet amassed as much research as the bovine dairy industry. Limited overseas research is available, but it may not be generalisable to New Zealand farms with their particular environments, pastoral and

seasonal systems, and breeds. Mastitis is recognised as one of the most impactful animal health challenges for dairy cows in New Zealand, but we lack information on the impact and management of mastitis on dairy sheep farms. Overseas, mastitis in dairy sheep is known to affect animal wellbeing and mortality, milk quantity, quality, and processing (Jaeggi *et al.* 2003; Leitner *et al.* 2004; Alba *et al.* 2019).

The incidence of clinical mastitis in New Zealand dairy flocks is unclear. French researchers noted in a review that the incidence within a dairy sheep lactation or year was typically less than 5% and comprised largely of sporadic cases, though larger-scale outbreaks had been reported (Bergonier and Berthelot 2003). In 1968, a New Zealand cross-sectional survey of non-dairy ewes, in which 19,427 ewes were manually examined in either August-September or January-February, reported signs of clinical mastitis and udder defects in 1.7% of the ewes (Quinlivan 1968a).

While the incidence may be low compared to New Zealand dairy cows, among which the incidence was estimated to be 10.0% among cows calving between 8 July and 21 August 1997 (McDougall 1998) and 14.8% for the entire lactation (Petrovski *et al*. 2009), clinical mastitis in dairy sheep can often be severe. Veterinary examinations of mastitic ewes in a Norwegian study of dairy sheep found moderate or severe systemic signs in half of the 509 ewes detected with clinical mastitis, gangrene in 9% of glands, and pyrexia in more than half of affected ewes (Mork *et al.* 2007).

Understanding the aetiology is essential for controlling mastitis. Anecdotally, many New Zealand dairy sheep farmers have conducted individual investigations into mastitis for their own farms, but, to our knowledge, the aetiology has not been systematically studied using a consistent methodology.

Little specific information exists on the treatment of clinical mastitis in sheep. There are no intramammary products registered for lactational or dry ewe therapy in New Zealand. Several injectable antimicrobials are registered for sheep, with only a few stating a milk withholding period, so, in many instances, long milk withholding periods of 35 days (the default withholding period for the off-label use of medicines in New Zealand) are applied, limiting the economic value of treatment due to the prolonged milk discard period.

In view of the above-mentioned lack of published data, the objective of this study was to describe the incidence, aetiology, treatment, and outcomes of farmer-reported clinical mastitis on New Zealand dairy sheep farms.

4.3 Materials and methods

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

4.3.1 Study design, setting and participants

This was a prospective cohort study conducted on 20 New Zealand commercial sheep milking farms, commencing on each farm's seasonal start of spring lambing (July to September 2022) and ending when the last ewe was dried off (February to May 2023). The aim was to recruit a mixture of farms (including some who supplied processors and others who processed their own milk) across different regions. Farms were convenience selected based on these criteria, and also the willingness of farmers to participate and comply with the study procedures. Farmers were contacted through the milk processing companies or directly (independent operators). The 20 study farms were located in the Waikato (n=12), Wairarapa (n=2), and Canterbury (n=6) regions. All farms lambed entirely in the spring except for one farm that sold fresh milk and also had a smaller autumn-lambing flock. All ewes present on

the enrolled farms that lambed in the 2022-2023 season were eligible for inclusion in the study.

4.3.2 Study procedures

Farmers were contacted by phone or email approximately fortnightly to December 2022 to provide updates, arrange sample processing, assess protocol compliance, and answer any questions.

4.3.2.1 On farm procedures

All clinical mastitis diagnoses and procedures were conducted by farm staff. Prior to the start of lambing, the first author met all study farm owners/managers to introduce the study, train them on the definition of clinical mastitis, specimen collection, and storage and record keeping. Farms were provided with a kit that contained forms and a folder for collecting information, specimen jars, paper towels for preparing teats, disposable gloves, marker pens, alcohol-impregnated teat wipes, and two digital thermometers. Farms were given written protocols and summaries on the definition of clinical mastitis, when to look for clinical mastitis, what to do when clinical mastitis is detected, how to collect milk samples aseptically, and a summary of the study protocol (supplementary materials). In person and online meetings were conducted to provide training in mastitis diagnosis and sample collection to farm managers and staff. A smart phone application was developed for participating farm staff, which included tools for capturing case, treatment, and outcome information, electronic copies of the written study resources, and a video on how to aseptically collect a milk specimen. The application can be accessed at https://www.jotform.com/app/221517759848874. More information is in the Appendix (10.4).

Briefly, farm staff were instructed to record information on each clinical mastitis case detected on the farm, collect duplicate milk samples from the affected gland(s), and manage the case according to their usual methods. No case management advice or direction was provided. Farmers were asked to enter treatment (if applicable) and outcome details once the animal's outcome was known. If these details were not collected, the first author recovered as much information as possible from farm records at in-person interviews at the end of the 2022-2023 season, when farm demographic and management information was also collected, or through email or telephone communications.

Clinical mastitis was defined as a change in the appearance of milk (clots, blood, watery milk, and/or red milk) and/or signs of inflammation in the gland (swelling, uneven udder, pain, lumps, or discharging sores). The case definition was intentionally pragmatic and designed for farmer detection under commercial milking conditions. Milk abnormalities and gross gland changes, including swelling, pain, asymmetry, lumps and discharge, were included because they are readily observable during routine milking. Redness was excluded because it may reflect superficial skin inflammation rather than mammary gland inflammation, while heat was excluded because it is difficult to assess consistently by touch under winter and spring conditions. Events not considered to be clinical mastitis were: positive rapid mastitis test (RMT) (any reaction as judged by the farmer) without clinical signs (i.e., subclinical mastitis), blood in the milk without signs of inflammation, and conditions limited to the skin, such as warts and parapox virus lesion (orf). Farmers were requested to manually examine all ewes for clinical mastitis at the first time they were handled during the seasonal lambing period (e.g., at lambing or at first milking), by stripping milk from both glands and visually examining and palpating the udder for inflammation. Farmers were not directed to specifically examine ewes for clinical mastitis at any other time

but were asked to report all cases of clinical mastitis that were identified during the usual running of their farms.

For every case of clinical mastitis, staff were required to enter, when known, 1) information about the ewe (identification, age, lambing date, litter size at pregnancy diagnosis, litter size at birth, first milking date, whether it rained on two or more days in the week before clinical mastitis was detected, whether the ewe lambed indoors or outdoors, and number of ewes being milked on the date of diagnosis); 2) case details (date of clinical mastitis detection, identification of the affected glands, presence of clots in the milk, colour of the milk, whether a drop in milk yield was noticed in the two days prior to diagnosis, whether the gland was painful, swollen, or uneven, whether there were lumps in the gland, whether the gland was gangrenous, the ewe's rectal temperature, and whether the ewe was depressed); and 3) whether they intended to treat the case. Gangrene was defined as the gland being cold to the touch or sloughing. Depression was defined as lethargy, inappetence, or being unable to stand. Staff could also upload photographs and any other files they deemed relevant.

Staff were required to enter treated ewes' identification, the product(s) used, the dose, frequency, number of treatments, route, and site of administration, milk and meat withholding periods, and any other details or files they deemed relevant.

For all ewes diagnosed with clinical mastitis, staff were required to select one of the following outcomes: 1) still milking: full recovery without lasting effects; 2) still milking: recovered but with lasting effects (e.g., weak gland); 3) dried off: full recovery without lasting effects; 4) dried off: recovered but with lasting effects (e.g., weak gland); 5) culled: due to lasting mastitis problems; 6) culled: due to non-mastitis problems; 7) died: due to mastitis; 8) died: due to non-mastitis problems; or 9) other outcome (describe). Staff were prompted to describe any lasting problems. Dates of drying off, culling, death, or putting out

with lambs were required when relevant, and the date of the outcome entry was recorded for all cases.

Information entered via the app was automatically collated into a spreadsheet online and downloaded for analysis, with separate spreadsheets for case, treatment, and outcome information.

Upon diagnosis and prior to any treatment, farm staff collected two milk samples (3-10 ml) from affected glands using aseptic technique (supplementary materials). As soon as possible after collection, but not later than three hours, the samples were stored in the farmers' freezers. Frozen samples were picked up at planned visits described in Chapters 3, 5 and 6 and at other times if study personnel were nearby (i.e., a maximum of three months after farmer diagnosis) and transported on ice back to the first author's laboratory. One sample per case was shipped on ice to Massey University, Palmerston North, New Zealand, for microbiology using insulated boxes with ice, packed so the jars were stabilised upright, and kept at -20°C until analysed. The second (duplicate) sample was retained at the first author's laboratory and kept at -20˚C.

4.3.2.2 Laboratory procedures

The microbiological procedures aimed at identifying bacteria that grow in aerobic conditions, because aerobic culture is the standard, practical method used in mastitis investigations and is aligned with how farmers and veterinarians would approach the problem in practice. Isolation of *Mycoplasma* spp was not attempted because it requires specific culture conditions or molecular methods and was outside the scope of this baseline study. In dairy sheep, *Mycoplasma* spp are primarily important as causes of contagious agalactia, for which there is no evidence of occurrence in New Zealand (Jay and Tardy 2019). In addition, the broader clinical syndrome associated with contagious agalactia, including arthritis,

keratoconjunctivitis, pneumonia and septicaemia, was not reported by participating farmers. Frozen milk samples were allowed to thaw at room temperature. Thawed samples were swirled and 10 μL of milk were aseptically collected and deposited as a drop on a quarter of a 5% sheep blood agar plate (Fort Richard, Auckland, New Zealand) and spread using a sterile spreader. Plates were incubated aerobically at 35-37°C for 40-48 hours. After incubation, plates were inspected for bacterial growth and the number of colony types recorded. Plates with three or more colony types were defined as contaminated and not analysed further. For samples with one or two colony types, the number of colonies was recorded for each type. A minimum of one colony type with three or more colonies was necessary for the plate to progress to the next stage, except where a colony morphologically resembled *Staphylococcus aureus*, when only one colony was required (Gonzalo *et al.* 2019). 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*), one isolated colony was picked and subcultured onto a new 5% sheep blood agar plate, and incubated as above to generate an isolate. The isolates were submitted for matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF, Microflex LT Biotyper, Bruker Daltonics, Billerica, MA) for bacterial identification at Epicentre, Massey University, Palmerston North, New Zealand. A protein extraction protocol was used, in which 1 µL of colonies were added to 300 µL of HPLC-grade water and suspended by vortexing in 900 µL of ethanol, centrifuged, and the pellet dried.

For cases 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 if it was not contaminated or the isolates were unidentifiable by MALDI-TOF.

The bacteriology results were categorised into six groups: 1) growth of *Streptococcus uberis*; 2) growth of *S. aureus*; 3) growth of non-aureus staphylococci (NAS); 4) growth of "other" bacteria (i.e., *E.coli*, *Arthrobacter gandavensis*, enterococci, or *Klebsiella*); 5) no growth or unidentifiable bacterium; 6) mixed growth (two colony types); and 7) contaminated samples.

4.3.3 Statistical analysis: sample size calculation and data analysis

The number of commercial dairy sheep farms in New Zealand was unknown when this study was developed. It was estimated that at the start of the 2022/2023 milking season there were approximately 40 commercial farms. A target of 20 farms (approximately 50% of New Zealand dairy sheep farms) was set. Assuming a whole-season farm-level incidence of clinical mastitis of 1.7% (Queiroga 2017), a mean farm size of 750 ewes and 50% of farms having 250-1500 ewes (a standard deviation of 312.5 ewes), and an intraclass correlation coefficient (ICC) of clinical mastitis of 0.04 (Compton *et al.* 2007), a sample size of 255 cases from 20 farms allowed the incidence of clinical mastitis to be estimated with 95% confidence (α =0.05) and a precision of ±1.25%.

Raw data were collated from the app website (case, treatment, and outcome data), farmer interviews (missing and updated case, treatment, and outcome data), and microbiology results, and imported into RStudio 4.2.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) for statistical analysis.

The data were collated and merged in wide format by uniquely identifying each case, gland, and ewe on each farm, then examined for completeness, duplications, consistency, and spurious values. Cases occurring within 21 days of a previous case in the same gland, or if the side of the gland in the first or second case was unknown, were assumed to be the same case, and were excluded. The 21-day interval was selected pragmatically because no universally accepted dairy-sheep-specific recurrence interval was available. In a review and

meta-analysis of clinical mastitis recurrence in dairy cows, published recurrence definitions varied substantially, with reported minimum intervals between clinical mastitis episodes ranging from 1 to 30 days; among studies requiring more than 24 hours, the minimum interval ranged from 5 to 30 days (Jamali et al. 2018). Pyrexia was defined as a rectal temperature ≥ 40.0 °C (Mork *et al.* 2007). Days in milk (DIM) at mastitis detection was calculated as the absolute difference in days between the lambing date and the date of mastitis detection. Mastitis treatments were categorised based on the active ingredient(s) of each product used. If more than one product was administered concurrently for a case, and only one antibiotic was used, the antibiotic was deemed the primary treatment. If more than one antibiotic was administered in series (none were administered in parallel), the first antibiotic to be administered was deemed the primary treatment. When animals were known to have died but it was unknown if they died or were culled, and for what reason, the outcome was defined as unknown. If microbiology identified two colony types, the MALDI-TOF results were reported for both. When the best and second-best MALDI-TOF matches identified different species for a single colony type, the best match was reported. When ewes had clinical mastitis in both glands and there were MALDI-TOF results available for each gland, both were reported.

The incidence of clinical mastitis was analysed in two ways with different numerators: 1) only the cases for which at least some case, treatment, outcome, and/or microbiology data were collected; and 2) the above cases but also cases that the farmers were aware of but for which no data were collected. For both methods, the denominator was the estimated total number of ewes that entered the milking flock in the 2022-2023 season.

Exploratory data analysis included generating tables of summary statistics and distributional plots, overall and by farm. Relationships between pairs of variables were visualised with frequency tables and plots. Pairwise associations between categorical variables were tested by

χ^2 analysis unless expected counts were <5, in which case Fisher's test was used. Unless otherwise stated, all statistical tests were two-tailed and the critical significance level was set at 5%.

To provide overall estimates of the incidence of clinical mastitis that were adjusted for clustering of clinical mastitis risk within farm, generalised linear regression models were constructed with a random intercept for farm for both methods of estimating the incidence described above. Both models contained only an intercept. Population-average predicted probabilities (i.e., the marginal predicted probabilities across all farms) and their 95% CIs 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 intra-class correlation coefficient (ICC), a measure of within-farm correlation (the proportion of variance that is at the farm level), was calculated for clinical mastitis by simulation to adjust for uneven cluster sizes (Goldstein *et al.* 2002). Farm-level incidence was calculated directly from the raw data. Because of the presence of 0% incidence on 4/20 farms, the confidence intervals were calculated using a Bayesian method with a Jeffreys prior of Beta (0.5, 0.5).

To compare the prevalence of clinical signs (clots, pain, swelling, uneven udder, lumps in the udder, gangrene, depression, and fever), separate logistic regression models were constructed to assess the relationship between each clinical sign (dependent variable) and, as separate predictor variables, (a) cases that were pyrexic and non-pyrexic; (b) cases that were depressed and not depressed, and (c) cases that were culture-positive and culture-negative. In all models, farm was included as a fixed effect to account for clustering.

4.4 Results

The median peak number of ewes milked per farm at any one time point over the study was 790 (range: 171-1,530), while the total was approximately 815 (range: 171-1,530). On two farms, more ewes were brought in when others were dried off, so the total number milked over the season was larger than the peak. All ewes lambed outdoors except on one farm, which lambed all hoggets (one-year-old ewe lambs) and ewes bearing three or more lambs indoors, and on two farms, which brought some ewes indoors during inclement weather (typically ewes bearing three or more lambs).

After removing nine observations deemed to be the same case as a previous observation from the same ewe, partial or complete data were available for a total of 236 cases from 221 ewes on 16 farms. No cases were observed on two farms. On another two farms, clinical mastitis cases were observed but no data were collected for any cases, while on another seven farms the farmers collected at least some data for some but not all cases. Treatment and outcome information was available for 109 and 199 cases respectively. Microbiology results were available for 160 glands from 135 cases from 135 ewes. On one farm, the staff accidentally collected milk samples from both glands of 16 ewes instead of just the affected glands, so up to 16 microbiology samples from non-clinically mastitic glands were included in the microbiology data (it is unclear if the ewes had mastitis in one or both glands).

Partial case information (n=204/236) arose from cases for which not all information was known by the farmer at the time of mastitis detection (n=52/236), and cases with at least some case information that were retrospectively added to the dataset at the farmer interviews at the end of the season (n=152/236). Cases without treatment information (n=127/236) were recorded as not treated. Fifteen farmers stated that they did not treat all cases, while five stated that they did not treat any cases. Cases without recorded outcomes (n=37/236) were

ewes that were culled or died but the reason was unknown (n=9/236) or that could not be located at the end of the study (n=28/236). Missing microbiology data (n=101/236) arose from recorded cases with at least partial case information but from which no milk samples were collected (n=10/236), and cases with at least partial case information that were retrospectively added to the dataset (n=91/236).

Of the 164 cases with lambing location information, 17 (7%) ewes lambed indoors. Reports indicated it had rained on two or more days in the week prior to detection for 53 (62%) of the 85 cases with information. The median (min, max) age at lambing was 3 (1, 8) years for the 219 cases with a known age. Median (min, max) litter sizes were 2 (1, 4) and 2 (1, 4) lambs at scanning and birth respectively for the 110 and 102 cases with that information respectively.

4.4.1 Incidence and timing of clinical mastitis across the lactation

The data on the incidence of clinical mastitis across the entire lactation are presented using only the cases for which at least some case data were collected either during the study or at the farmer interviews (Figure 1A), and using the same data but adding the cases that were thought or known to have occurred but for which no case data or milk samples were collected (Figure 1B). These extra cases arose from farmer recollection or farm records. Farmers removed lambs from ewes and started milking the ewes within 10 days after lambing on 14 farms (six of which removed lambs within 48 hours of birth), while lambs were reared on the ewes for >10 days (4 to 12 weeks) after parturition on six farms. In the latter group, the first milking (and observation in the milking parlour) was therefore delayed. Those farms are indicated with asterisks in Figures 1A and 1B After excluding cases occurring within 21 days of the previous case in the same gland (or when one or both glands were not recorded), a

second case was recorded in 15 ewes and a third case in one ewe.

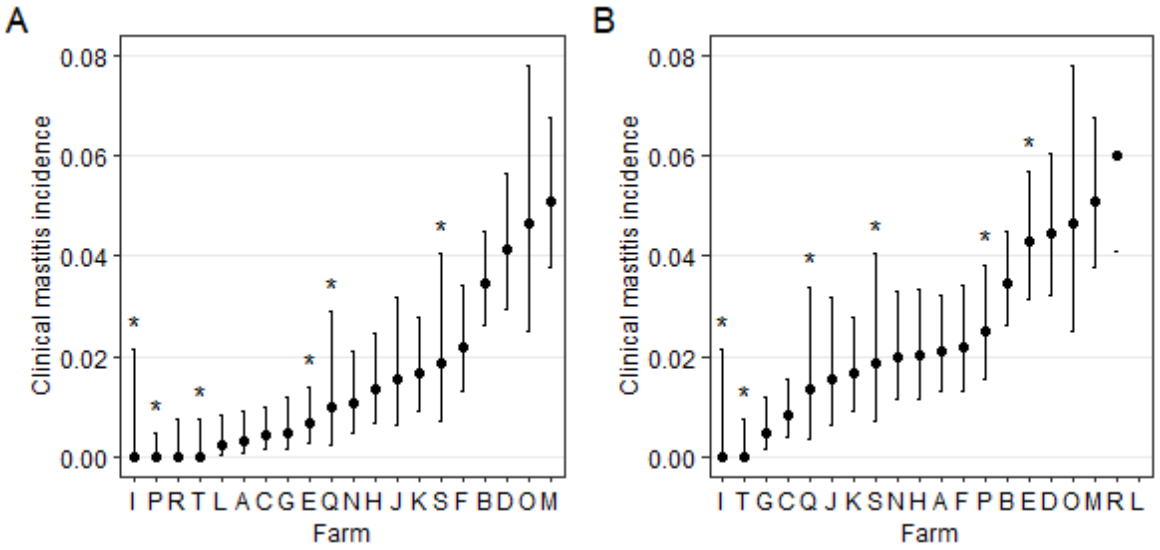

Figure 1: Incidence of farmer-reported clinical mastitis on 20 dairy sheep farms in the 2022-2023 season, A) based on data collected during the study, and B) based on farmer estimates at the end of the season (there is no farmer-estimated clinical mastitis incidence for farm L because the number of cases was unknown). Vertical lines represent 95% confidence intervals. Asterisks indicate farms on which the first milking was delayed until at least 10 days postpartum because lambs were reared on the ewe.

Across all study farms, the overall clinical mastitis incidence over the lactation period was 1.8 (95% CI =1.0-3.2) %, ranging from 0 to 5.1% at the farm level based on the data collected during the study. The incidence was 0% on four farms, two of which did not collect clinical mastitis data for the study (but these two farms did report cases retrospectively and therefore did not have 0% incidence when including cases for which no case data or milk samples were collected), and the other two diagnosed no cases. For the latter two farms, one of those farms selected milking ewes from a larger conventionally farmed flock at weaning and screened out ewes with any udder defects prior to first milking, unlike the other 19 farms, while the second farm also practiced delayed first milking but had a dedicated milking flock and did not screen ewes. Including cases for which no case data or milk samples were collected, the overall clinical mastitis incidence over the lactation period was 2.3 (95% CI =1.6-3.3) %, ranging from 0 to 6% at the farm level.

Using only the cases for which study data were collected, clinical mastitis cases were reported between 14 June 2022 and 30 March 2023, with most (139/236; 59%) cases occurring in August to October 2022 (i.e., during the lambing period). Of the cases with known lambing and mastitis detection dates (n =199), mastitis occurred at a median (IQR) of 7 (3, 40) days in milk (Figure 2). Farmers detected 19/199 (10%) of the cases prior to the ewes' recorded lambing dates. For cases detected on or after the recorded date of lambing (n=180), the median (IQR) number of days between lambing and clinical mastitis detection was 11 (4, 46) days.

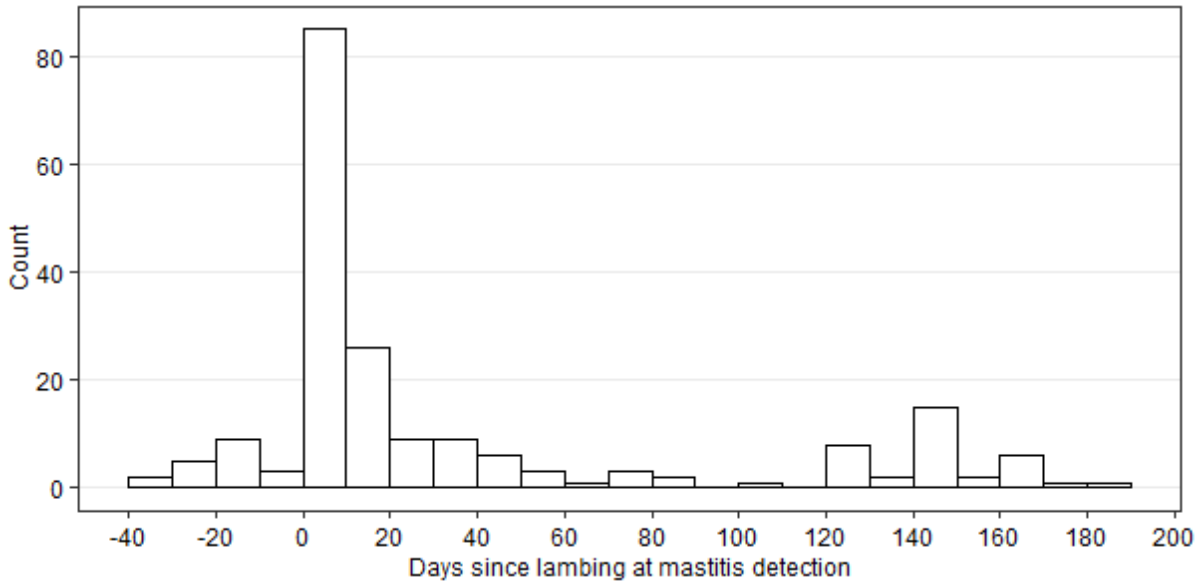

Figure 2: Timing, in relation to lambing, of farmer-reported clinical mastitis cases on 20 dairy sheep farms in the 2022-2023 season.

Clinical mastitis incidence was not strongly clustered within farms, with ICCs of 0.052 using only cases with study data, and 0.017 cases for which no case data or milk samples were collected respectively.

4.4.2 Clinical features

More cases were detected in the right gland than the left gland and the majority of cases featured clots in the milk, swelling, and unevenness (Table 1). The prevalence of some

clinical features varied between pyrexic and non-pyrexic ewes, and depressed and non-depressed ewes (Table 2). Pyrexia and depression were associated, with 60% of depressed ewes being pyrexic, compared to 13% of non-depressed ewes. Ewes that were depressed were also more likely to have swollen glands (79%) than ewes that were not depressed (48%).

Table 1: Clinical features of farmer-reported clinical mastitis cases (n=236) on 20 New Zealand dairy sheep farms in the 2022-2023 season.

Variable	Prevalence[1]
Affected gland(s)	
Both	11/143 (7.7%)
L	58/143 (41%)
R	74/143 (52%)
Clots observed in milk	55/94 (59%)
Gland was painful	41/92 (45%)
Gland was swollen	51/93 (55%)
Udder was uneven	68/96 (71%)
Lumps in gland	36/92 (39%)
Mastitis was gangrenous[2]	6/93 (6.5%)
Ewe was depressed[3]	24/92 (26%)
Ewe was pyrexic	19/76 (25%)

[1]Denominators differ due to incomplete data collection on farm.

[2]The gland was cold to the touch or sloughing.

[3]The ewe was lethargic, inappetent, or unable to stand.

Table 2: Associations between the presence of pyrexia (rectal temperature ≥ 40.0 °C) and depression (lethargy, inappetence, or inability to stand) with other clinical features of farmer-reported clinical mastitis cases on 20 New Zealand dairy sheep farms in the 2022–2023 season.

	Presence of pyrexia			Presence of depression		
Variable	**Pyrexic, (n=19)[1]**	**Not pyrexic, (n=57)[1]**	**p-value[2]**	**Depressed, (n=24)[1]**	**Not depressed, (n=68)[1]**	**p-value[3]**
Clots observed in milk	12/19 (63%)	33/57 (58%)	0.423	18/24 (75%)	37/68 (54%)	0.947
Gland was painful	10/18 (56%)	23/56 (41%)	0.374	19/22 (86%)	22/68 (32%)	0.143
Gland was swollen	13/19 (68%)	28/56 (50%)	0.340	19/24 (79%)	32/67 (48%)	0.025
Udder was uneven	15/19 (79%)	37/56 (66%)	0.843	22/24 (92%)	42/67 (63%)	0.426
Lumps in gland	8/19 (42%)	22/55 (40%)	0.894	14/23 (61%)	22/67 (33%)	0.145
Mastitis was gangrenous[4]	2/19 (11%)	3/56 (5.4%)	0.460	6/24 (25%)	0/67 (0%)	0.997
Ewe was depressed[5]	12/19 (63%)	8/56 (14%)	0.010			
Ewe was pyrexic				12/20 (60%)	7/55 (13%)	0.010

[1]Denominators differ due to incomplete data collection on farm.

[2]From logistic regression model.

[3]From separate logistic regression models for each variable with farm included as a fixed effect.

[4]The gland was cold to the touch or sloughing.

[5]The ewe was lethargic, inappetent, or unable to stand.

4.4.3 Treatment

Tylosin was the most commonly used primary treatment (Table 3). Most injections were administered intramuscularly and 49/52 (94%) of intramuscular injections were administered in the rump, while 35/37 (95%) of subcutaneous injections were administered in the neck. Meloxicam was used in addition to antibiotics in five cases. For the cases with known milk withholding periods, farmers applied a 3-day milk withholding period for 46/49 (94%) of cases in which tylosin was used, and 35 days for the remaining three cases. Milk withholding periods of three (n=4), five (ewes milked twice daily) or 10 (ewes milked once daily) (n=11), or 35 (n=10) days were applied for oxytetracycline. The single ewe treated with procaine penicillin had a milk withholding period of 35 days applied. Milk withholding periods of 30 (n=1) and 35 days (n=13) were applied to ewes treated with penethamate hydroiodide.

Table 3: Treatment protocols reported by farmers for clinical mastitis cases (n=109) on 20 New Zealand dairy sheep farms in the 2022-2023 season.

Variable	Prevalence[1]
Route	
IM	52/91 (57%)
PO	2/91 (2.2%)
SC	37/91 (41%)
Site	
Mouth	2/91 (2.2%)
Neck	38/91 (42%)
Rump	51/91 (56%)
Milk withholding period applied (days)[2]	
0	2/91 (2.2%)
3	50/91 (55%)
5 (TAD) or 10 (OAD)	11/91 (12%)
30	1/91 (1.1%)
35	27/91 (30%)
Meat withholding period applied (days)	
0	2/89 (2.2%)
7	2/89 (2.2%)
10	48/89 (54%)
14	11/89 (12%)
21	17/89 (19%)
30	1/89 (1.1%)
35	8/89 (9.0%)
Primary treatment[3]	
Immune support bolus	2/109 (1.8%)
Oxytetracycline injection	37/109 (34%)
Penethamate hydroiodide injection	14/109 (13%)
Procaine penicillin injection	1/109 (0.9%)
Tylosin injection	55/109 (50%)

[1]Denominators differ due to incomplete data collection on farm.

[2]OAD: milking frequency = once a day, TAD: milking frequency = twice a day.

[3]If more than one product was administered concurrently, and only one antibiotic was used, the antibiotic was deemed the primary treatment. If more than one antibiotic was administered in series (none were administered in parallel), the first antibiotic to be administered was deemed the primary treatment.

4.4.4 Final case outcomes

Farms varied in their approach to clinical mastitis, but case management was largely applied at the farm level. For example, some farmers frequently attempted treatment, while others instantly dried affected ewes off and placed them in a separate paddock until they could be

sent for slaughter, though some ewes were treated for welfare reasons. The most common outcome was being instantly dried off to be culled without treatment, followed by still milking and recovered with lasting problems (Table 4). Only 30/199 (15%) of ewes with outcome information had no lasting problems (and were either still milking at the time the outcome was assessed or had been dried off for other reasons).

Table 4: Reported outcomes (n=199) for farmer-diagnosed clinical mastitis cases (n=223) on 20 New Zealand dairy sheep farms in the 2022-2023 season.

Outcome	Prevalence
Culled: due to lasting mastitis problems	23/199 (12%)
Culled: due to non-mastitis problems	1/199 (0.5%)
Died: due to mastitis	4/199 (2.0%)
Dried off: full recovery with no lasting problems	13/199 (6.5%)
Dried off: recovered with lasting problems	2/199 (1.0%)
Euthanised	2/199 (1.0%)
Instantly dried off to be culled without treatment	63/199 (32%)
Other	21/199 (11%)
Put out with lambs (removed from milking flock)	4/199 (2.0%)
Still milking: full recovery with no lasting problems	17/199 (8.5%)
Still milking: recovered with lasting problems	49/199 (25%)

Of the 21 ewes with an outcome of "other", eight died or were culled for uncertain reasons, six were instantly dried off without treatment but not culled even though they were not intended to be milked next season, two remained in the flock but it was unknown if they were healthy or if the affected gland had become non-functional (either deliberately by the farmer or not) (a "1-titter"), two had an unknown outcome, one was instantly dried off and expected to be milked next season, one ewe's affected gland was no longer functional and probably culled, and one was kept but the udder status was unknown.

When known (n=67 cases), lasting problems included: the gland became dry or retained only one functional gland (n = 47), the gland became gangrenous, went solid and ruptured, or sloughed (n = 6), the gland milked poorly with or without elevated somatic cell count (n = 5),

treatment failure (n = 3), the gland became an aerobic plate count risk (n = 2) where, following microbiological testing by the farmer, the ewe was deemed to be a risk for an elevated bulk milk aerobic plate count, the gland gave a positive milk culture (n = 2), the gland became solid (n = 1), or hard with abdominal swelling in the ewe (n = 1).

4.4.5 Microbiological results

Nearly half (75/160, 46.9%) of all the milk samples submitted were culture-negative (Table 5). Secondary milk samples were processed for microbiology for 10 cases due to the primary sample being contaminated (n=6) or having an unidentifiable growth (n=4). Of the uncontaminated culture-positive samples, only 7/76 (9%) grew 3-10 colonies and 69/76 (91%) grew >10 colonies. *Strep. uberis* and *S. aureus* were the most common species identified (22/160 and 18/160 respectively), and NAS accounted for 19/160 (12%) of isolates.

Table 5: Aetiology of farmer-diagnosed clinical mastitis cases (n=160 glands from 135 cases on 16 farms) in a prospective study of clinical mastitis on 20 New Zealand dairy sheep farms in the 2022-2023 season.

Aetiology	Prevalence
No growth	75/160 (47%)
Streptococcus uberis	22/160 (14%)
Staphylococcus aureus	18/160 (11%)
Contaminated	9/160 (5.6%)
Unidentifiable	7/160 (4.4%)
Escherichia coli	5/160 (3.1%)
Staphylococcus simulans	5/160 (3.1%)
Staphylococcus warneri	4/160 (2.5%)
Staphylococcus chromogenes	3/160 (1.9%)
Staphylococcus haemolyticus	3/160 (1.9%)
Acinetobacter pseudowolffii & *Streptococcus uberis*	1/160 (0.6%)
Arthrobacter gandavensis	1/160 (0.6%)
Enterococcus faecalis	1/160 (0.6%)
Enterococcus villorum	1/160 (0.6%)
Klebsiella oxytoca	1/160 (0.6%)
Staphylococcus borealis	1/160 (0.6%)
Staphylococcus devriesei	1/160 (0.6%)
Staphylococcus haemolyticus & *Staphylococcus warneri*	1/160 (0.6%)
Streptococcus uberis & *Enterobacter ludwigii*	1/160 (0.6%)

There were clear differences between farms in aetiology, though 5/16 of the farms with bacteriology data had <5 isolates (Figure 3). *Strep. uberis* was isolated on 12/16 farms, *S. aureus* on 8/16 farms, NAS on 8/16 farms, and both *S. aureus* and NAS on 2/16 farms. On the 15 farms with microbiology data for more than one case, *Strep. uberis* was identified in more than one ewe on five farms and *S. aureus* in more than one ewe on three farms.

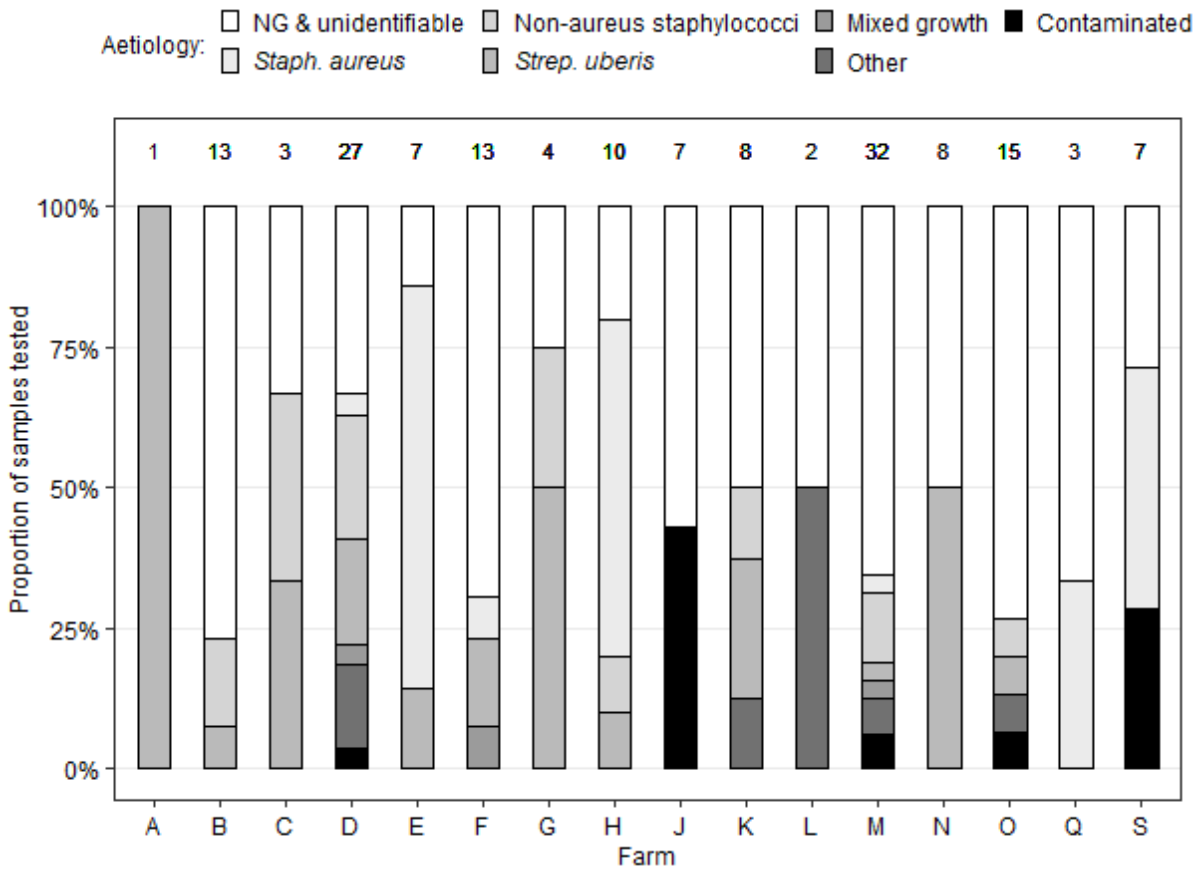

Figure 3: Aetiology of farmer-reported clinical mastitis cases (n=69), excluding contaminated, unidentifiable, and culture-negative samples, on 20 dairy sheep farms in the 2022-2023 season. Number above bars represent the number of isolates for each farm. Other bacteria include *E.coli*, *Arthrobacter gandavensis*, *Enterococcus faecalis*, and *Klebsiella oxytoca*.

Swelling was reported in 14/38 (36.8%) and 30/44 (68.2%) culture-negative and culture-positive cases respectively (p = 0.006). The prevalence of other clinical features did not differ significantly between culture-negative and culture-positive cases. For ewes with complete clinical observation data, 23/32 (71.9%) of ewes with both swelling of the affected gland and

clots in the milk returned a bacteriologically-positive milk sample, compared to 21/51 (41.2%) of ewes with neither clots nor swelling ($p = 0.025$).

4.5 Discussion

This is the first systematic study of clinical mastitis on New Zealand dairy sheep farms to our knowledge. We aimed to include all cases of clinical mastitis on the study farms, avoiding the bias that can occur when relying on milk samples collected spontaneously by farmers, who may be more likely to sample certain cases. This study provides evidence-based baseline parameters, collected from about one half of the commercial farms existing at the time of the study. The farms were not randomly selected, so selection bias cannot be excluded. However, purposive recruitment aimed to improve industry coverage by including both farms supplying large processors and independent operators across a range of regions.

Nearly all of the ewes with clinical mastitis lambed outdoors, consistent with the management of ewes overall on the study farms. Rain was reported on at least two days in the week prior to lambing for most cases, typical of New Zealand's spring weather. Data were not collected for ewes that did not have a case of clinical mastitis, so no inferences can be made about the effect of lambing indoors, weather conditions, age, and litter size on the risk of clinical mastitis.

Farmer-reported clinical mastitis cases may produce different results to the capture of cases by single investigators, mainly due to a higher risk of deviations from the protocol by the farmers. Farmers and their staff may have made diagnostic or data entry errors, or failed to collect information or milk samples, especially during the extremely busy lambing period (when most clinical mastitis cases occurred), causing the incidence to be underestimated. Furthermore, apart from at the first milking, we did not direct farmers to routinely screen ewes during milking, which likely means the true incidence was underestimated and the

severity overestimated (since farmers may be more likely to detect cases that are more clinically severe). Unfortunately, it was not logistically feasible to send investigators to capture all the cases on 20 geographically dispersed farms. To reduce the amount of error due to non-compliance with the protocol, we provided standardised training to the farm personnel prior to the study and monitored the study periodically. There was a 0.5% absolute difference (a 28% relative difference) in the incidence of clinical mastitis over the lactation period between what was estimated from the cases with at least some case information 1.8 (95% CI =1.0-3.2) % and the farmers' retrospective estimates after the study had finished 2.3 (95% CI =1.6-3.3) %. However, while the difference between the two estimates is large in relative terms, the estimated incidence of farmer-reported clinical mastitis was low using either method.

The mean clinical mastitis incidence over the lactation period of 1.8 (95% CI =1.0-3.2) % is consistent with overseas estimates: 0.9% in Portugal (Queiroga 2017), and 1.7 cases per 100 ewe-months in Jordan (Lafi *et al.* 1998). However, the three studies differed methodologically. Queiroga (2017) made a point estimate (i.e., a prevalence estimate), which is likely to be lower than the incidence across a season or lactation. Lafi *et al.* (1998) visited farms monthly, so the incidence may be underestimated. The incidence in the present study was similar to the 1.7% point prevalence estimated by Quinlivan (1968a) in non-dairy New Zealand sheep, but the latter study shares the same limitations as Queiroga (2017). Compared to dairy cows, the clinical mastitis incidence in New Zealand dairy sheep appears to be low. Given the early stage many dairy sheep farms are at in New Zealand, it is possible that the incidence of clinical mastitis will change over time as the industry evolves due to shifts in management and genetics.

Repeat cases occurring within 21 days were excluded from the analysis if they were in the same gland or the gland in either case was unknown but were included otherwise. However,

many ewes were dried off or culled at the first case, so it is not clear what the recurrence rate would be if all ewes were retained, and this should be considered when advising farmers on expected recurrence rates.

The present study was not designed to describe the duration and sequelae of clinical mastitis cases. Reliable estimation of these outcomes would require prospective follow-up with standardised udder examinations before or at diagnosis and at defined intervals afterwards, allowing pre-existing udder morphology to be distinguished from mastitis-associated changes and enabling consistent assessment of clinical resolution, persistent gland changes, recurrence, culling and longer-term production effects. The prompt removal of ewes observed on many farms in this study meant that duration was often irrelevant. New Zealand researchers examined non-dairy ewes (n=48) weekly over six weeks for udder hardness and lumps and showed that chronic udder changes in ewes can persist for several weeks at least (Zeleke *et al.* 2021).

The between-farm incidence range over the lactation period (0-5%) was relatively small compared to similar work on New Zealand dairy cows. Petrovski *et al.* (2009) measured the lactation incidence in 16 Northland herds and found an incidence range of 4-33%, and McDougall (1998) found a range of 1-21%. There is very little information on between-farm variation in incidence for dairy sheep worldwide. Queiroga (2017) reported the point prevalence of clinical mastitis in Portuguese dairy flocks to range from 0% to 8%, which is not very different from our range.

Clinical mastitis was not strongly clustered within farms, with ICCs of 0.052 using only cases with study data, and 0.017 cases for which no case data or milk samples were collected. In practical terms, this means that ewes within the same farm were only slightly more similar in their clinical mastitis risk than ewes from different farms, with most variation occurring

within farms rather than between farms. Thus, although farm-level management remains relevant, these findings suggest that industry-level prevention should include attention to ewe- and gland-level risk factors, rather than focusing solely on differences between farms. We could not locate any published ICC values for clinical mastitis in dairy sheep. Compton *et al.* (2007) found a similarly low ICC of 0.04 for New Zealand dairy heifers.

Two farms in the present study did not report cases during the study period. One of those farms did not have a dedicated milking flock, and instead chose the desired number of ewes from a larger, conventionally farmed flock on the same property at weaning, and screened out ewes with udder defects. In addition to the absence of mastitis monitoring at milking until weaning, the screening applied prior to entry to the milking flock may have reduced the risk of clinical mastitis by eliminating ewes with mastitis risk factors (that have already caused them to suffer mastitis and develop udder defects), potentially explaining the 0% incidence over the lactation period. The other farm also delayed milking until weaning and therefore may also have missed cases occurring between lambing and first milking. In both cases, it is not possible to determine if clinical mastitis cases were occurring after the first milking but being missed, or if they were both low-incidence flocks due to breeding and management practices. Despite delayed first milking potentially reducing the sensitivity of clinical mastitis detection, there were some farms that commenced milking within 10 days of lambing with relatively low clinical mastitis incidences, and farms that delayed first milking with higher incidences (Figure 1).

Clinical mastitis occurred mostly during lambing and within 14 days of parturition, with some cases seen prior to the recorded lambing date. There is little published information on the timing of clinical mastitis in dairy sheep. In a Norwegian prospective study, 20% of clinical mastitis cases were diagnosed in the first two days of lactation (Mork *et al.* 2007). A true increase in incidence around lambing is biologically plausible. In seasonal, pasture-based

New Zealand dairy cattle, early lactation is a recognised high-risk period for clinical mastitis (McDougall 1998), and the periparturient period is associated with metabolic and immunological changes that may increase susceptibility to intramammary infection (Khan *et al*. 2023). In dairy ewes, lambing also coincides with increased exposure of the teat canal to bacteria from lambs, wet environments and contaminated udder skin, while suckling may cause teat-end trauma (Cooper *et al*. 2023). Detection intensity may have influenced the apparent timing of cases, either through heightened awareness during lambing or underdiagnosis during periods of heavy workload. However, the observed temporal pattern is consistent with clinical mastitis risk being genuinely highest during lambing and early lactation, indicating that prevention and detection efforts should be maximised during this period.

Visible changes to the udder, such as lumps, swelling, or asymmetry, were common among mastitic ewes. A number of published studies have measured udder defects (Quinlivan 1968a; Ridler *et al.* 2021), but fibrosis and abscesses are not necessarily reflective of new cases. Many of the changes in the present study could have been the sequelae of mastitis in previous seasons. Udder defects have been shown to persist across lactations. Zeleke *et al.* (2023) conducted a longitudinal study, in which the udder halves of 991 non-dairy ewes were assessed four times annually over two years. Ewes with udder defects (hardness or lumps) diagnosed at a pre-mating visit had relative risk ratios of having an udder defect later in the same season or at the pre-mating visit in the following season ranging between 6.8 to 1,444, demonstrating the persistence of udder defects.

The lower prevalence of swelling among culture-negative cases suggests that some farmers may be diagnosing chronic udder defects as clinical mastitis, some of which may be the result of previous inflammation, or confusing colostrum for mastitis (though cases were defined as having other signs of inflammation, farmers may not have always followed the case

definition). Combining the presence of clots in the milk and swelling of the affected gland did not improve upon swelling alone in terms of estimating the prevalence of culture-negative milk samples. It is therefore challenging to differentiate between active cases of clinical mastitis and sequelae of previous cases on clinical signs alone. Prospective studies recording the presence of deformities at lambing before the majority of clinical mastitis occurs could help to clarify this point, as well as farmer training and development of a diagnostic pathway. Developing a dry gland (either deliberately by the farmer or not) was the most common lasting problem reported in the present study after a case of clinical mastitis. Many such ewes may present with uneven or otherwise abnormal glands in the following season, and therefore be mistaken as having clinical mastitis.

Negative cultures ("no growth") was the most common microbiological result in this study, occurring in 75/160 (47%) cases. Mistaking udder defects for clinical mastitis may have contributed to the high proportion of negative cultures, as well as the up to 16 samples collected from non-mastitic glands. Initially, a conservative value of ≥ 10 colonies was considered in the present study as the threshold for a sample to proceed to aetiological diagnosis, to align with previous work on ovine milk microbiology (Fthenakis 1994; Lafi *et al.* 1998; Vasileiou *et al.* 2018). However, a more liberal threshold of ≥ 3 colonies was eventually applied, so it appears unlikely that the colony count threshold was responsible for the high prevalence of negative culture results observed in our study. No growth occurred in 13% and 19% of cases in the studies of Mork *et al.* (2007) and Queiroga (2017) respectively. For non-dairy ewes diagnosed with an udder deformity by Ridler *et al.* (2021), 47% were culture-negative, using a similar microbiological method.

Another possible explanation for the high prevalence of negative cultures is the freezing and thawing of milk samples. However, data on the effect of freezing on pathogen's viability in sheep milk are scarce and ambiguous. Smith *et al.* (2011) compared the recovery rate of

bacteria in 50 ewe milk samples known to be infected after freezing for four or eight weeks, with or without preservation with glycerol. The proportion of samples that were bacteriologically positive declined by up to 50% with time across all pathogens regardless of preservation. They found that the lower the CFU count of the sample, the more its viability was impacted by freezing. Samples with CFU counts >100/ml on day 0 yielded maximum reductions of 25% in isolation after freezing. For culture-negative samples on day 0, freezing increased the isolation rate for *S. aureus*. Sanchez *et al.* (2003) demonstrated that freezing milk samples from goats infected with subclinical mastitis at -20°C or -80°C up to 730 days increased the CFU count of coagulase-negative staphylococci (CNS) (*Staphylococcus caprae*, *Staphylococcus epidermidis*, *Staphylococcus chromogenes* and *Staphylococcus xylosus*) with time and reduced the CFU count of Gram-negative bacilli (*Serratia marcescens*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*) 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 weeks reduced the number of samples that had cultures of *E. coli* or *Actinomyces pyogenes*, increased the number of samples that had cultures of CNS, and had no effect on streptococci and *S. aureus*. In their study of clinical mastitis in dairy ewes, Mork *et al.* (2007) submitted some samples immediately for culture and froze other samples, but no comparison of the results for fresh and frozen samples was presented. Lafi *et al.* (1998) and Queiroga (2017) submitted all samples immediately for culture, while Ridler *et al.* (2021) froze all samples. The limited data from sheep suggest freezing reduces the viability of some bacterial species in milk, though this may be more pronounced in low CFU count samples, and deterioration appears to be greater for Gram-negative pathogens. The samples were collected from ewes diagnosed by farmers as having clinical mastitis, and therefore may have higher CFU counts than samples from randomly selected healthy ewes or ewes with subclinical mastitis, meaning the effect of freezing may

have been less pronounced. However, we cannot rule out an effect of freezing on the high prevalence of no growth samples, particularly in the case of Gram negative pathogens. In future studies, the use of pathogen PCR and/or enzymatic markers of inflammation could help differentiate active from chronic cases and culture-negative Gram-positive from Gram-negative cases.

There is little information on the clinical features of sheep mastitis. Mork *et al.* (2007) reported "moderate or severe" systemic signs in 49% of ewes, but the severity categories were not defined in detail. In the present study, unevenness was the most common observation (71% of cases). Depression and pyrexia occurred in 26 and 25% of cases respectively, with overlap between clinical signs. Mork *et al.* (2007) diagnosed pyrexia in 56% of cases using the same definition (i.e., ≥40.0 ˚C) in our study. We observed gangrene in 6.5% of ewes, similar to the 8.8% reported by Mork *et al.* (2007). The frequent reports of pyrexia, depression, and gangrene indicate that, while clinical mastitis in dairy ewes may be less common than is typical for dairy cows under New Zealand conditions, it can often be severe and should be addressed promptly for animal welfare and productivity reasons. Depression is a somewhat subjective measure but appears to be associated with the severity of the mastitis and may be a useful observation for farmers to use. Severity scoring systems have been developed for dairy cows (Wenz *et al*. 2006); future work could adapt these for ewes.

Tylosin was the most common treatment (50% of treated cases) recorded in our study. It is unclear if that is due to a genuine or perceived high efficacy or favourable registration conditions compared to other products. Many farmers however did not treat ewes with clinical mastitis at all, with only 109/236 (46%) of cases recorded as being treated. The use of anti-inflammatory drugs (meloxicam) was only reported in five cases. The 25% prevalence of pyrexia, and the association between pyrexia and depression, suggest that treatment with non-

steroidal anti-inflammatory drugs could be a useful part of a dairy ewe clinical mastitis treatment toolkit, especially on farms where such ewes are not immediately culled. Non-steroidal anti-inflammatory drugs have been shown to increase feeding time, improve rumination, reduce pain, oedema, fever and somatic cell count, and improve fertility in dairy cows (Li *et al*. 2023).

Full recovery without lasting problems was only reported for 8.5% of the ewes. The most common problem (when recorded) was developing a dry gland or being deliberately made into a ewe with only one functional gland. Even those ewes with an outcome of "still milking (full recovery)" (i.e., no lasting problems) may have deteriorated subsequently, meaning the probability of a full recovery was even lower. The likelihood of recovering without lasting problems is unclear for dairy cows, but clinical failure (as defined by the farmer) among New Zealand dairy cows diagnosed with clinical mastitis due to a range of pathogens was 13.4% when treated with an intramammary product containing amoxicillin, clavulanic acid, and prednisolone twice daily for five treatments (McDougall *et al.* 2019). Other authors have discussed the low probability of complete recovery and the objective of treatment being to avoid death and cull the ewe (Bergonier and Berthelot 2003). In a review of mastitis in sheep, Watson and Buswell (1984) commented that "The course of the disease is rapid and its effects severe, so much so that treatment is invariably directed at saving the life of the ewe in the full knowledge that changes to her affected udder tissue are irreversible". It is not possible to draw inferences between outcomes and clinical presentation or treatment choice because treatment and management were largely applied at the farm level (e.g., a farm used only one treatment, or all mastitic ewes were dried off instantly without treatment). When developing treatment plans, veterinarians should set realistic expectations on the probability of ewes making full recoveries from an udder health and productivity perspective and remain conscious of protecting the ewe's welfare while attempting treatment.

Overall, the aetiology was predominantly Gram positive, with *Strep. uberis*, *S. aureus*, and NAS the most common isolates. Staphylococci have been repeatedly shown as the most common cause of clinical mastitis in dairy sheep overseas (Bergonier and Berthelot 2003; Mork *et al.* 2007; Queiroga 2017) and were the most common bacteria isolated from ewes with udder defects among non-dairy sheep in New Zealand (Quinlivan 1968b; Ridler *et al.* 2021). However, *S. aureus* and NAS were only isolated on 50% and 44% of farms that submitted milk samples for culture, suggesting some clustering of aetiology by farm. In contrast, *Strep. uberis* appeared more frequently than in previous work from overseas. It accounted for 14.0% of cases in the present study, while it accounted for 1.6% of cases in the study of Mork *et al.* (2007), was not found in the studies of Queiroga (2017) or Lafi *et al.* (1998), and only accounted for 1.9% of samples from non-dairy ewes with udder defects in the study of Ridler *et al.* (2021). A higher contribution by *Strep. uberis* to clinical mastitis for New Zealand dairy ewes, compared to New Zealand non-dairy ewes and dairy ewes in other countries, may be a feature of our predominantly outdoor, spring-lambing systems. This is consistent with New Zealand dairy-cattle literature, wherein *Strep. uberis* is a common cause of clinical mastitis in seasonal, pasture-based herds and is particularly associated with environmental exposure around calving. In a Northland study of 14 New Zealand dairy farms milking a total of 3,765 cows, Petrovski *et al.* (2009) isolated *Strep. uberis* from 23.3% of cases of clinical mastitis. *Strep. uberis* caused the majority of dry period clinical mastitis cases and new intramammary infections during the dry period among New Zealand dairy cows with late lactation somatic cell counts <200,000 cells/mL (Woolford *et al*. 1998). In a study of primiparous New Zealand cows, Compton *et al*. (2007) isolated *Strep. uberis* from 64% of clinical mastitis cases. Future dairy-sheep studies should therefore investigate whether risk factors and preventive measures identified in New Zealand dairy cattle are relevant to ewes, including lambing-paddock hygiene, stocking density around lambing,

udder and teat contamination, wet underfoot conditions, teat-end condition, early-lactation surveillance, and targeted interventions to reduce environmental exposure to *Strep. uberis* before and during lambing. Intervention studies could evaluate whether management strategies aimed at reducing teat-end contamination before and during early lactation, such as paddock selection and housing, reduce *Strep. uberis* clinical mastitis in dairy ewes.

Cases were categorised as *Strep. uberis*, *S. aureus*, NAS, "other", "no growth or unidentifiable", mixed growth, and contaminated to provide a high-level summary of the aetiology and compare aetiology between farms. In the original classification, cases that were unidentifiable by MALDI-TOF (n = 7/160, 4.4%) were grouped with culture-negative cases (n = 75/160, 46.9%), giving n = 82/160 (51.3%) in the "no growth or unidentifiable" category. However, because these unidentifiable samples yielded bacterial growth, it is more appropriate to group them with "other" isolates. Reclassification would increase the "other" category from n = 9/160 (5.7%) to n = 16/160 (10.0%) and leave "no growth" as n = 75/160 (46.9%). This reclassification does not alter the main findings: nearly half of submitted samples were culture-negative, and *Strep. uberis*, NAS and *S. aureus* remained the predominant identified isolates among culture-positive cases.

4.6 Conclusions

Farmer-reported clinical mastitis affected 0-6% of ewes at the farm level across the 2022-2023 season, with an overall lactation incidence of 1.8 (95% CI =1.0-3.2) % using the study data, or 2.3 (95% CI =1.6-3.3) % using study data and farmer estimates. However, 46.9% of cases were culture-negative, and culture-negative cases were less likely to have swelling of the gland, suggesting the incidence might have been overestimated. Cases occurred predominantly over the lambing period and within the first 14 days of lactation. Pyrexia and depression were reported in 25 and 26% of ewes respectively, suggesting a role of supportive

treatment for systemic signs of clinical mastitis. Clots in the milk and swelling and unevenness of the glands were recorded in the majority of cases. Pyrexia and swelling were more likely among ewes that were clinically depressed, and clots and swelling swelling was more prevalent among glands that returned culture-positive milk samples. Most ewes did not recover without lasting problems such as a dry gland. *Streptococcus uberis* (14%), non-aureus staphylococci (12%), and *Staphylococcus aureus* (14, 12, and 11% of cases with microbiology data) were the most common isolates.

4.7 Acknowledgements

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5 SUBCLINICAL MASTITIS IN NEW ZEALAND DAIRY EWES 1: PREVALENCE AND RISK FACTORS

The previous chapter addressed clinical mastitis, which can be observed directly by farmers because affected ewes have altered milk, visibly inflamed glands, and/or are generally unwell (sometimes seriously). The next two chapters turn to subclinical mastitis, a more challenging and complex subject because it is not observable with the naked eye and requires screening tests such as the somatic cell count, rapid mastitis test, bulk milk monitoring, and milk culture.

The first of the two chapters, Chapter 5, focuses on subclinical mastitis *per se* by defining it, reporting its prevalence, characterising the bacterial causes, briefly summarising somatic cell count and rapid mastitis test, and identifying risk factors. The second, Chapter 6, extends Chapter 5 by exploring diagnostic tests for subclinical mastitis and whether it is linked to elevated aerobic plate count (a measure of overall bacterial load in milk).

These two chapters were published as companion articles in the Journal of Dairy Science and are in press at the time this thesis was written. Chapter 5 can be accessed at https://doi.org/10.3168/jds.2025-27075.

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

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5.1 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 approximately 15 randomly selected ewes on each of 20 dairy sheep farms at early, mid, and late lactation. California mastitis tests (measured on a scale of 0, trace, 1, 2, and 3) and aerobic bacterial culture were performed at the gland level, and somatic cell count (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 a SCC >500x10^3 cells/mL and/or a California mastitis score ≥1. 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 1 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 *S. 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 three visits. The prevalence was low compared to 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 because, although farm-level management remains relevant, these findings suggest that industry-level prevention should include attention to ewe- and gland-level risk factors, rather than focusing solely on differences between 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 with 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 while 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.

Keywords: sheep; milk quality; mastitis; somatic cell count.

5.2 Introduction

Compared to 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. There were an estimated 30,000 ewes being milked on approximately 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.* 2016), 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.

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 a positive milk culture, usually in the presence of elevated somatic cell count (SCC) (Fthenakis 1994; Lysitsas *et al.* 2024) and/or California mastitis test (CMT) (Las Heras *et al.* 1999) and/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 *S. 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 and 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.

5.3 Materials and Methods

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

5.4 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 (Anonymous 2020), and given the rapid expansion, it was estimated that at the start of the 2022/2023 milking season there were approximately 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. Because a two-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):

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

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 three 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% confidence interval) 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 (teat and udder morphology and pathology assessments were undertaken at the same time). Enrolling 15 ewes per visit from 20 farms was calculated to have a precision of 6.8%.

5.4.1 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.* 2025a). 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 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 three farms, which lambed selected ewes indoors (e.g., ewes bearing triplets, one-year-old ewes, or other ewes during bad weather).

Visits were planned on 3 occasions on each farm during the 2022/2023 lactation season: August - October 2022 (visit 1), November - 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 hours after the morning milking to avoid prolonging milking time, except for 1 farm, where ewes were examined prior to 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 they were 1) under treatment or had been treated within the previous 30 days for illness; 2) were diagnosed with clinical mastitis on the day of sampling (defined as visual or palpable udder changes with clots in the milk); 3) 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 and/or feces that the operator deemed it unlikely that milk samples would be uncontaminated. However, no ewes presented with any of these exclusion criteria.

5.4.2 Study procedures

5.4.2.1 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*. (2025a). In brief, for the first two 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 p^{th} 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 prior to the visit. For all other visits, on the day prior to 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*. (2025a). 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), and "hard" (7), and teat palpation scores were collapsed into "normal" (1) and "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 four-point scale on farm and subsequently

categorized into three 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 one 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 approximately 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. CMT was measured on a 5-point scale (negative, trace, 1, 2, 3) as described by Schalm *et al.* (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 hours 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).

5.4.2.2 Producer interviews

When known, ewe demographic information (age, number of fetuses at pregnancy diagnosis, number of lambs born, lambing date, and first milking date) was collected from the farm

owners/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 the supplemental materials (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 only milked once daily during the drying off process at the end of lactation (~1 week), it was deemed to have milked twice daily all season.

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)
Lambing to lamb removal interval (days)
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 22-23 season
Number of milking staff - early lactation
Number of milking staff - mid-late lactation
Number of seasons the farm has operated (including 22/23 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)

5.4.2.3 Laboratory procedures

Somatic cell count was measured at the ewe level and aerobic bacterial culture at the gland level. 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 35-37°C for 40-48 hours. 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 analyzed further. 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 above to generate an isolate. The isolates were identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (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.

5.4.3 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, 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 year of age at lambing), 2-tooths (2 years of age at lambing), and mixed age (older than 2-tooths). Between-visit differences in lambing date, age at lambing, lambing to first milking interval, body condition score, and days since lambing at the visit were tested with the Kruskal-Wallis rank sum test, while the Fisher 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 one 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 two teats or glands except for teat length and width, for which the median was used. If inflammation or lesions were diagnosed in at least one teat or gland, the ewe was deemed positive.

Descriptive statistics were calculated for SCC and it was categorized into "normal" (≤500 x 10^3 cells/ml), intermediate (between 500 x 10^3 and 1 x 10^6 cells/ml), and "high" (>1 x 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 isolates), and the prevalence of each was calculated and plotted by farm.

5.4.3.1 Subclinical mastitis prevalence

Subclinical mastitis was defined at the ewe level as a bacteriologically positive milk sample (as defined above) in 1 or both glands alongside a CMT score ≥1 in the same gland and/or SCC >500 x 10^3 cells/ml (Fragkou *et al.* 2014). A bacteriologically positive milk sample with no increased CMT score (<1) or SCC <500 x 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 ≤500 x 10^3 cells/mL) and intermediate or high SCC, low (CMT <1) and high CMT, and positive and negative culture were presented as a table 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 intra-class correlation coefficient (ICC, the proportion of variance that was 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 datasets, 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 SDs clustered around the model residual SD.

5.4.3.2 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 above. Between-farm differences were tested with the Fisher exact test.

5.4.3.3 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, two-tooth, and mixed-age), BCS (1-2 and 2.5-4), udder depth, separation, suspension and teat placement scores (1-2, 3, and 4-5), gland palpation (normal and abnormal), teat end hyperkeratosis (group 1 and groups 2-3), number of lambs born (1, 2, ≥3), interval between lambing and lamb removal (<7, ≥7 days), 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), their associations with categorical variables were tested with the Chi square test or, if expected counts in any cell were <5, the Fisher 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 square test or Fisher 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 backwards 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 two models was compared by calculating the area under the receiver-operating characteristic curve (AUC) for each model and testing the difference with the method described by de Long *et al*. (1988).

5.5 Results

5.5.1 Enrolment and data

Across the three visits, 893 observations were made on 882 unique ewes. Eleven ewes were examined at two visits by chance; however, three of these lacked ear tags and may, in fact, represent six different untagged ewes. No ewes were excluded at the selection stage. Visits 1-3 were conducted from 24 August to 6 October 2022, 7 November to 22 December 2022, and 25 January to 16 March 2023 respectively. Outside of the first three 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 and/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*. (2025a), and the reasons for missing data are summarized in the supplemental materials.

5.5.2 Farm and ewe information

Because ewes were randomly selected anew at each visit, there were between-visit differences in the lambing spread of the selected ewes, with median lambing dates of 07 August (range = 17 July - 19 September), 26 August (range = 09 July - 16 October), and 22 August (range = 02 July - 15 October) for visits 1, 2, and 3 respectively (Kruskal-Wallis p <0.001). Of the 409 ewes with data on the number of lambs born, there were 138 (34%), 211 (52%), 57 (14%) and 3 (0.7%) ewes with singles, twins, triplets, and quadruplets, respectively. These proportions did not differ between visits (Fisher 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).

Table 2: Median and range 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 (years)	659	2 (1, 7)	3 (1, 7)	2 (1, 7)	2 (1, 7)	0.010
Lambing to first milking interval (days)	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.

5.5.3 Somatic cell count

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 - 34,953,000 cells/ml. There were 748, 53, and 89 samples with normal (<500 x 10^3 cells/mL), intermediate (between 500 x 10^3 and 1 x 10^6 cells/mL), and high (>1 x 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.

5.5.4 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).

5.5.5 Aerobic culture results

Milk samples from 1,763 glands from 884 ewes were submitted for aerobic culture. MALDI-TOF was performed on 103 isolates 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. MALDI-TOF 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). *S. 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 ID 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 & *Staphylococcus warneri*	1 (<0.1%)
Staphylococcus warneri & *Staphylococcus epidermidis*	1 (<0.1%)
Streptococcus ovis	1 (<0.1%)

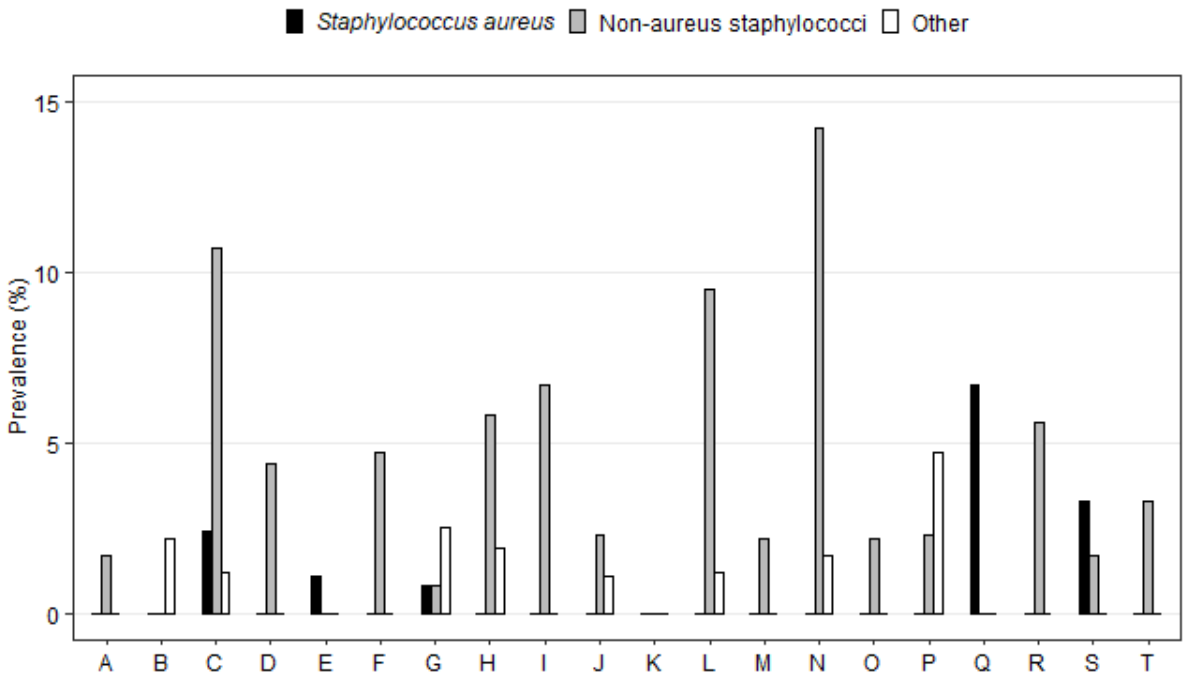

Figure 1: Percentage of milk samples on each farm that were confirmed as non-aureus staphylococci, *S. aureus*, and other species (Other), in a study of udder health of randomly selected ewes (n=873) on 20 commercial dairy sheep farms in New Zealand.

5.6 Subclinical mastitis

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

Table 4: Bacterial milk culture, somatic cell count (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.

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

[1]Dichotomized into 'low' (≤500,000 cells/mL) and 'high' (>500,000 cells/mL)

[2]Dichotomized into 'negative' (0 or trace) and 'positive' (1-3)

We diagnosed subclinical mastitis in 58/856 (6.8%) ewes, and mammary carriage (a bacteriologically positive milk sample with CMT <1 and SCC <500 x 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 1-3 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 =0.102).

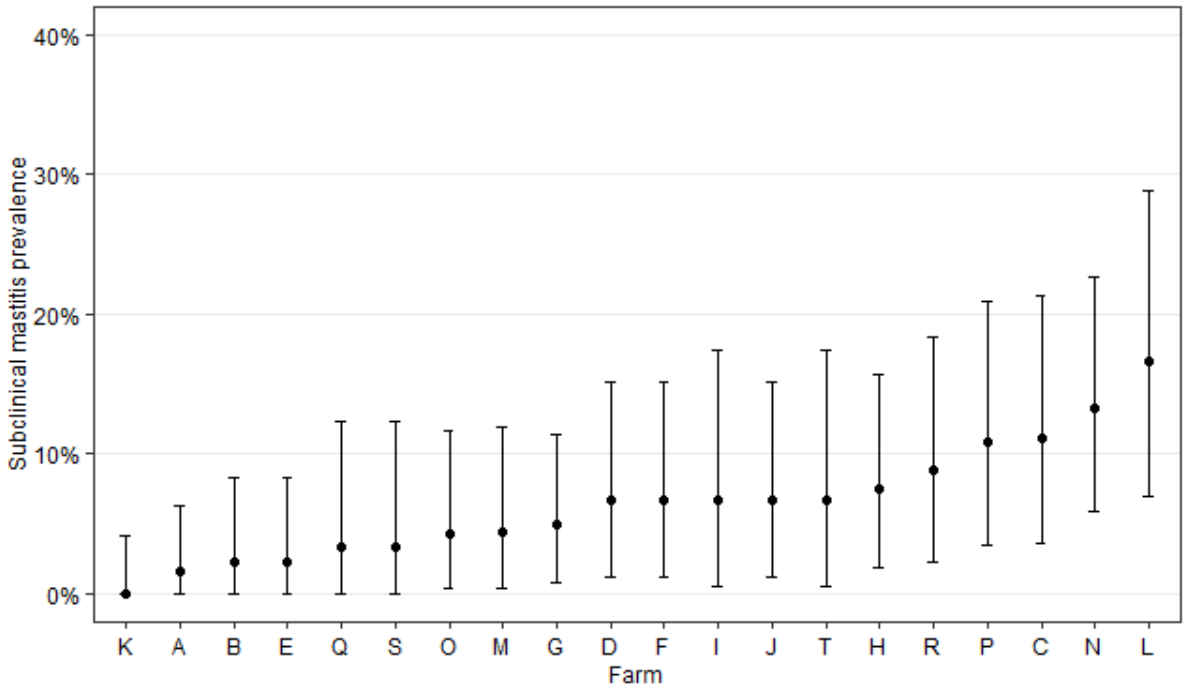

Figure 2: Prevalence of subclinical mastitis on each farm in a study of udder health of randomly selected ewes (n=871) on 20 commercial dairy sheep farms in New Zealand. Error bars represent 95% confidence intervals.

5.6.1 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*. (2025a). 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 the supplemental materials.

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

Variable	N	Overall N = 856	Negative N = 798	Positive N = 58	p-value[1]
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]Wilcoxon rank sum test

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

Variable	N	Overall N = 856	Negative N = 798	Positive N = 58	p-value
Visit	856				0.12[2]
Visit 1		266	242 (91%)	24 (9.0%)	
Visit 2		306	285 (93%)	21 (6.9%)	
Visit 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[1]	846				0.028[3]
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[3]
Group 1		837	782 (93%)	55 (6.6%)	
Group 2 or 3		9	6 (67%)	3 (33%)	
No. seasons the farm has operated (including 22/23 season) - categorized	856				0.2[2]
2-3		476	439 (92%)	37 (7.8%)	
4-25		380	359 (94%)	21 (5.5%)	

[1]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); and group 3 = severe hyperkeratosis (both teat ends ≥ 3).

[2]Pearson's Chi-squared test

[3]Fisher's exact test

Positive subclinical mastitis associations (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 twice daily all season (compared to 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 to 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 to high line), and teat spray use for the whole season (compared to 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/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/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 to 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 symmetry 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.

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.

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

[1]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); and group 3 = severe hyperkeratosis (both teat ends ≥ 3).

Abbreviations: CI = Confidence Interval, OR = Odds Ratio, NA

5.7 Discussion

Our goal was to establish a baseline dataset 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 to European studies, though 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 in prevalence (but not the direction of change over time) 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 and/or a SCC >500 x 10^3 cells/mL in the present study) as well as altered neutrophil and lymphocyte 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 prior to culture, which may have altered bacterial viability. The limited published research on the effect of freezing on culture of ewe milk suggests freezing reduces the viability of some bacterial species in milk, though this may be more pronounced in low CFU count samples (Smith *et al.* 2011), and deterioration appears to be greater for Gram-negative pathogens (Sanchez *et al.* 2003).

We did not sample all ewes on each farm, so random error may have contributed to the low prevalence. While 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 there was 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 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 there are no registered dry ewe therapies or mastitis vaccines for sheep in New Zealand, removing these two factors as competing explanatory elements in this dataset. 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 years, possibly because more than half of the ewes were on farms in the first 2-3 seasons of production. The age of ewes in other published studies has 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 0-11.4% among first and second parity ewes across the three visits, but 5.5-22.2% for third and greater parity ewes. The lower prevalence in the present study may reflect the younger age of the ewes, but age was not included in the final model, though age data were only available for approximately 75% of ewes.

A between-farm difference was not confirmed. With an ICC of 0.04, there was little clustering of subclinical mastitis prevalence within farm, 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, such as teat end damage, and variation within farms (e.g., monitor 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*. Ten *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. *Strep. 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 USA found *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.

Cases were categorised as *S. aureus*, NAS, and "other" to provide a high-level summary of the aetiology and compare aetiology between farms. In the original classification, cases that were unidentifiable by MALDI-TOF (n = 6/1763, 0.3%) were grouped with culture-negative cases (n = 1650/1763, 93.6%), giving n = 1656/1763 (93.9%) in the "no growth or unidentifiable" category. However, because these unidentifiable samples yielded bacterial growth, it is more appropriate to group them with "other" isolates. Reclassification would increase the "other" category from n = 16/1763 (0.9%) to n = 22/1763 (1.2%) and leave "no growth" as n = 1650/1763, 93.6%). This reclassification does not alter the main findings: the

vast majority of submitted samples were culture-negative, and NAS and *S. aureus* remained the predominant identified isolates among culture-positive cases.

The correlation between SCC and RMT, and optimal SCC thresholds for diagnosing intramammary infection, are presented in Chambers *et al*. (2025b). 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, non-pathogenic infections, loss of bacterial viability, and delays in the return of SCC and CMT to normal values after infection resolution may hypothetically 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 three 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 only found 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 the low prevalence of subclinical mastitis and insufficient study power.

Teat end roughness and thickness are known risk factors for new intramammary infection with *S. aureus* in dairy cows (Zadoks *et al*. 2001). In dairy ewes, 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 to the 17.4% reported by Vouraki *et al*. (2018), which means that our estimate of the impact of teat end hyperkeratosis on the risk of subclinical mastitis has a wide CI (1.7 to 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, there are no similar published data on the association between udder asymmetry and subclinical mastitis in dairy ewes. A comparison with dairy goats is relevant because, as in dairy sheep, udder conformation traits may influence milking efficiency, residual milk, teat exposure, and susceptibility to intramammary infection. Margatho *et al*. (2020) showed that udder asymmetry in Serrana goats was associated with a higher SCC, supporting the plausibility of an association between udder asymmetry and udder health in small ruminants. However, direct comparison is limited by differences in species, production system, udder assessment method, and outcome definition.

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 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 off 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 x 10^6 cells/mL, the prevalence of subclinical mastitis increased from 4.5% to 16.9% across three visits performed over 8-11 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 dataset, 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 impact of lactation stage on the risk of subclinical mastitis in dairy ewes between our study and that 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 approximately 1 month 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 (see supplemental table S5), the lack of such an association does not rule out a potentially large association. For example, compared to 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 and/or CMT, and identified udder asymmetry and teat end hyperkeratosis as potent risk factors for subclinical mastitis. However, these two factors should not be used to make management decisions (such as treatment, drying off, or culling) alone, 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/or milk culture. When using SCC data alone, it is not clear what threshold to apply and how many SCC measurements are required to be confident that a ewe does or does not have subclinical mastitis. Chambers *et al*. (2025b) proposed a SCC threshold based on a single SCC measurement from the same dataset. Berthelot *et al*. (2006), however, proposed that at least two 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 2-3 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 impact on the conclusions drawn from this study.

Visits were made 2-3 hours after milking on all farms except 1, where they were made prior to 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 prior to 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 and udder measurements 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. While 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 findings may also be useful for other emerging or pasture-based dairy sheep systems. For example, the findings suggest that other emerging or pasture-based dairy sheep industries should validate SCC thresholds, mastitis prevalence estimates, and risk factors under their own production conditions, rather than assuming that values derived from established Mediterranean systems are directly transferable. 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, while udder asymmetry in dairy ewes has not been studied elsewhere to our knowledge. This suggests that while 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. For example, New Zealand dairy sheep farmers may need to combine contagious mastitis control, such as identifying and managing *S. aureus*-infected ewes, with pasture-specific environmental control measures, such as selecting drier lambing paddocks, avoiding heavily contaminated areas, monitoring teat-end condition, and intensifying detection during early lactation. The lower subclinical mastitis prevalence observed in New Zealand may also alter the economics of some interventions; for instance, if *S. aureus* vaccines become available for dairy sheep in New Zealand, their cost-effectiveness may be lower than in higher-prevalence systems unless targeted to higher-risk farms or ewes.

5.8 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 dairy ewes are not substantially different to those in more intensive systems. Subclinical mastitis was not strongly clustered within farms (ICC = 0.04), suggesting that prevention efforts should prioritize identifying and managing ewe-level risk factors and variation within farms, while recognizing that key farm-level practices also contribute to disease control. Teat end hyperkeratosis was a potent but rare risk factor for subclinical mastitis, while 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 in diagnosing and managing subclinical mastitis. Culling or treatment decisions should not be based on teat end hyperkeratosis or udder asymmetry without other information such as bacterial culture and/or 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.

5.9 Acknowledgements

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5.10 Notes

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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.

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5.12 Supplemental information

Supplemental Table 1: Questions and reasons for exclusion in the analysis of risk factors, in a study of subclinical mastitis of randomly selected ewes (n=894) on 20 commercial dairy sheep farms in New Zealand.

Question	Used in risk factor analysis?	Reasons for not using in risk factor analysis
Are automatic cup removers used	Yes	
Are automatic teat sprayers used	No	0/20 farms used automatic teat sprayers
Are inline milk meters used	Yes	
Are lambs removed from ewes with ≥3 lambs	No	Unable to categorise responses due to varying policies within the season and/or multiple conditions for separation
Average milk production per ewe in the previous season (kg MS)	No	Unknown on 14/20 farms
Average milk production per ewe in the previous season (L)	Yes	
Average milk production per Ha (kg MS/Ha/last season)	No	Unknown on 14/20 farms
Breed	No	Large number of breeds and crosses used across and within farms - could not be categorised
Calendar period over which ewes were milked	No	Unable to categorise responses due to different policies within flocks for different ewes
Did ewes lamb on same paddocks as previous season	No	Not deemed relevant
Do ewes lamb indoors or outdoors	No	All farms lambed wholly outdoors or only lambed some ewes indoors
Effective farm size (Ha)	Yes	
Flock selenium status (if known)	No	Unknown on 10/10 farms and status was adequate on the 10 farms that had data
Frequency of teat spray application	No	Lack of variation (all farmers who used teat spray applied after at every milking)
Information colleced by milk meters (if applicable)	No	Only 5/20 farms used inline milk meters and only volume was measured on 4/5 farms
Is an automated feeding system used	Yes	
Is teat spray used for at least some of the season? (Y/N)	Yes	
Lambing start date	No	Separate dates for hoggets and mixed-age ewes on 18/20 farms, and minimal variation between farms (2 July - September for mixed-age ewes) due to seasonal lambing in the spring
Lambing to lamb removal interval (days)	Yes	
Milking flock size at peak	Yes	
Milking frequency	Yes	

Question	Used in risk factor analysis?	Reasons for not using in risk factor analysis
Number of bails/positions in the parlor	No	Not deemed relevant
Number of flock milk recording tests in the 22-23 season	Yes	
Number of milking staff - early lactation	Yes	
Number of milking staff - mid-late lactation	Yes	
Number of seasons the farm has operated (including 22/23 season)	Yes	
Part of the season teat spray is used	Yes	
Selenium supplementation and method (if applicable)	No	19/20 farms supplemented selenium, often through multiple routes, and the responses could not be categorised
System pressure	Yes	
System pulsation frequency	Yes	
Teat spray type	Yes	
Temperature in the milk tank (°C)	No	Not deemed relevant
Total number of feed troughs available	No	Not deemed relevant
Total number of staff - early lactation	Yes	
Total number of staff - mid-late lactation	Yes	
Type of feed troughs available (individual/group)	No	Not deemed relevant
Type of flow line (high/low)	Yes	
Type of milking parlour (e.g., rotary, herringbone)	No	17/20 farms had herringbone sheds
Use of gloves in milking shed (Y/N/personal choice/other)	Yes	
Vaccines administered in last 12 months	No	Not deemed relevant (no mastitis vaccines)

Supplemental Table 2: Number of observations missing raw data, and the reasons, in a study of udder health of randomly selected ewes (n=894) on 20 commercial dairy sheep farms in New Zealand.

Variable	N missing	Reason
Ewe information		
Age	234	Unknown by farmer (n=234)
BCS	3	Not recorded (n=3)
Breed	302	Unknown by farmer (n=302)
Recorded no. lambs born	821	Unknown by farmer (n=821)
Recorded no. lambs at pregnancy scanning	484	Unknown by farmer (n=484)
Recorded lambing date	414	Unknown by farmer (n=414)
Recorded first milking date	454	Unknown by farmer (n=454)
Milk information		
Somatic cell count	3	Mislabelled samples (n=2), no sample (n=1)
CMT - left gland	16	Atrophic/non-lactating gland (n=8), result not recorded (n=7), no sample taken (n=1)
CMT - right gland	13	Atrophic/non-lactating gland (n=4), result not recorded (n=7), no sample taken (n=2)
Aerobic plate count	19	Quality control (n=10), no sample (n=6), mislabelled (n=2), spreader (n=1)
Microbiology result - left gland	12	Missing sample (n=9), mislabelled (n=2), no sample (n=1)
Microbiology result - right gland	11	Missing sample (n=8), mislabelled (n=2), no sample (n=1)

Supplemental Table 3: Mean (range) of continuous variables explored as potential risk factors for subclinical mastitis, 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. Denominators differ due to missing data for some variables.

Variable	N	Overall N = 856	Negative N = 798	Positive N = 58	p-value[1]
Age (yr)	633	2.6 (1-7)	2.6 (1-7)	2.9 (1-7)	0.3
Body condition score	853	2.4 (1-4)	2.4 (1-4)	2.4 (2-4)	0.7
Days in milk	464	108.3 (8-243)	108.6 (8-243)	104.0 (19-223)	0.7
No. lambs born	398	1.8 (1-4)	1.8 (1-4)	1.9 (1-3)	0.3
Ewe median teat length (mm)	852	27.4 (17-51)	27.4 (17-51)	27.5 (22-40)	0.9
Ewe median teat width (mm)	852	15.8 (7-29)	15.8 (7-29)	16.0 (10-25)	0.5
Farm area (Ha)	856	58.0 (11-160)	58.3 (11-160)	53.4 (11-160)	0.5
Peak number of ewes milked	856	756.4 (171-1,530)	757.3 (171-1,530)	743.4 (171-1,530)	0.6
Total number of staff - early lactation	856	3.8 (2-10)	3.8 (2-10)	3.6 (2-10)	0.2
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 - early lactation	856	2.3 (1-4)	2.3 (1-4)	2.3 (1-4)	0.6
Number of milking staff - mid-late lactation	856	2.2 (1-4)	2.2 (1-4)	2.1 (1-4)	**0.11**
Lambing to lamb removal interval (days)	796	9.6 (1-56)	9.7 (1-56)	8.9 (1-56)	0.3
Mean season milk production per ewe (L)	604	271.2 (210-400)	271.1 (210-400)	272.5 (210-400)	0.4
Pulsation frequency	737	138.1 (119-182)	138.0 (119-182)	139.4 (119-182)	0.6
System pressure (kPa)	812	38.4 (32-43)	38.4 (32-43)	39.1 (32-43)	**0.055**
No. seasons the farm has operated (including 22/23 season)	856	4.3 (2-25)	4.3 (2-25)	4.1 (2-25)	0.3
No. flock milk recording tests in the 22-23 season	856	1.3 (0-6)	1.3 (0-6)	1.5 (0-6)	0.6

[1]Wilcoxon rank sum test (values < 0.2 are in bold)

Supplemental Table 4: Distributions of categorical variables explored as potential risk factors for subclinical mastitis, 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. Denominators differ due to missing data for some variables.

Variable	N	Overall N = 856	Negative N = 798	Positive N = 58	p-value[1]
Visit	856				**0.12[3]**
Visit 1		266 (100%)	242 (91%)	24 (9.0%)	
Visit 2		306 (100%)	285 (93%)	21 (6.9%)	
Visit 3		284 (100%)	271 (95%)	13 (4.6%)	
Udder depth score	856				0.2[4]
1		0 (NA%)	0 (NA%)	0 (NA%)	
2		6 (100%)	5 (83%)	1 (17%)	
3		126 (100%)	120 (95%)	6 (4.8%)	
4		586 (100%)	541 (92%)	45 (7.7%)	
5		138 (100%)	132 (96%)	6 (4.3%)	
Udder suspension score	856				0.9[4]
1		36 (100%)	33 (92%)	3 (8.3%)	
2		241 (100%)	223 (93%)	18 (7.5%)	
3		332 (100%)	310 (93%)	22 (6.6%)	
4		176 (100%)	164 (93%)	12 (6.8%)	
5		71 (100%)	68 (96%)	3 (4.2%)	
Udder separation score	854				0.3[4]
1		23 (100%)	20 (87%)	3 (13%)	
2		281 (100%)	260 (93%)	21 (7.5%)	
3		367 (100%)	341 (93%)	26 (7.1%)	
4		156 (100%)	150 (96%)	6 (3.8%)	
5		27 (100%)	26 (96%)	1 (3.7%)	
Teat placement score	856				0.8[4]
1		0 (NA%)	0 (NA%)	0 (NA%)	
2		72 (100%)	69 (96%)	3 (4.2%)	
3		435 (100%)	403 (93%)	32 (7.4%)	
4		290 (100%)	270 (93%)	20 (6.9%)	
5		59 (100%)	56 (95%)	3 (5.1%)	
Udder symmetry	854				**0.002[3]**
Yes		528 (100%)	504 (95%)	24 (4.5%)	
No		326 (100%)	293 (90%)	33 (10%)	
Udder inflammation	851	14 (100%)	13 (93%)	1 (7.1%)	>0.9[4]
Udder lesions	847	32 (100%)	30 (94%)	2 (6.3%)	>0.9[4]
Udder palpation	856				0.3[4]
Normal		839 (100%)	783 (93%)	56 (6.7%)	
Lump		17 (100%)	15 (88%)	2 (12%)	
Teat inflammation	852	4 (100%)	4 (100%)	0 (0%)	>0.9[4]
Teat lesions	853	18 (100%)	17 (94%)	1 (5.6%)	>0.9[4]
Teat palpation	856				0.6[4]

Variable	N	Overall N = 856	Negative N = 798	Positive N = 58	p-value[1]
Normal		805 (100%)	749 (93%)	56 (7.0%)	
Abnormal		51 (100%)	49 (96%)	2 (3.9%)	
Teat end hyperkeratosis[2]	846				**0.028[4]**
Group 1		837 (100%)	782 (93%)	55 (6.6%)	
Group 2		6 (100%)	4 (67%)	2 (33%)	
Group 3		3 (100%)	2 (67%)	1 (33%)	
Age (yr categorized)	856				0.3[3]
Hogget		164 (100%)	157 (96%)	7 (4.3%)	
2-tooth		165 (100%)	151 (92%)	14 (8.5%)	
MA		527 (100%)	490 (93%)	37 (7.0%)	
Use of gloves during milking	856				0.8[4]
Compulsory/all		320 (100%)	297 (93%)	23 (7.2%)	
Optional/some		506 (100%)	472 (93%)	34 (6.7%)	
Not worn		30 (100%)	29 (97%)	1 (3.3%)	
Teat spray use	856				**0.13[3]**
All season		358 (100%)	341 (95%)	17 (4.7%)	
Part season		424 (100%)	389 (92%)	35 (8.3%)	
Not used		74 (100%)	68 (92%)	6 (8.1%)	
Teat spray type	782				0.6[3]
Chlorhexidine		608 (100%)	566 (93%)	42 (6.9%)	
Iodine		174 (100%)	164 (94%)	10 (5.7%)	
Milking frequency	856				**0.13[3]**
Twice daily		272 (100%)	248 (91%)	24 (8.8%)	
Twice daily for part of the season then once daily		406 (100%)	379 (93%)	27 (6.7%)	
Once daily		178 (100%)	171 (96%)	7 (3.9%)	
Automatic cup removers	856	313 (100%)	296 (95%)	17 (5.4%)	0.2[3]
Inline milk meters	856	199 (100%)	183 (92%)	16 (8.0%)	0.4[3]
Type of flow line (high/low)	856				**0.014[3]**
High		458 (100%)	418 (91%)	40 (8.7%)	
Low		398 (100%)	380 (95%)	18 (4.5%)	
Automatic feeding system	856	690 (100%)	642 (93%)	48 (7.0%)	0.7[3]
Udder depth score - categorized	856				0.3[4]
1-2		6 (100%)	5 (83%)	1 (17%)	
3		126 (100%)	120 (95%)	6 (4.8%)	
4-5		724 (100%)	673 (93%)	51 (7.0%)	
Udder suspension score - categorized	856				0.8[3]
1-2		277 (100%)	256 (92%)	21 (7.6%)	
3		332 (100%)	310 (93%)	22 (6.6%)	
4-5		247 (100%)	232 (94%)	15 (6.1%)	
Udder separation score - categorized	854				0.2[3]
1-2		304 (100%)	280 (92%)	24 (7.9%)	
3		367 (100%)	341 (93%)	26 (7.1%)	
4-5		183 (100%)	176 (96%)	7 (3.8%)	

Variable	N	Overall N = 856	Negative N = 798	Positive N = 58	p-value[1]
Teat placement score - categorized	856				0.7[4]
1-2		72 (100%)	69 (96%)	3 (4.2%)	
3		435 (100%)	403 (93%)	32 (7.4%)	
4-5		349 (100%)	326 (93%)	23 (6.6%)	
Udder palpation - categorized	856				0.3[4]
Normal		839 (100%)	783 (93%)	56 (6.7%)	
Abnormal		17 (100%)	15 (88%)	2 (12%)	
Teat end hyperkeratosis - categorized[2]	846				**0.019[4]**
Group 1		837 (100%)	782 (93%)	55 (6.6%)	
Group 2 or 3		9 (100%)	6 (67%)	3 (33%)	
Body condition score - categorized	853				0.7[3]
1-2		362 (100%)	336 (93%)	26 (7.2%)	
2.5-4		491 (100%)	459 (93%)	32 (6.5%)	
No. lambs born - categorized	398				0.4[4]
1		133 (100%)	126 (95%)	7 (5.3%)	
2		208 (100%)	189 (91%)	19 (9.1%)	
≥3		57 (100%)	52 (91%)	5 (8.8%)	
Lambing to lamb removal interval (days) - categorized	796				>0.9[3]
1-7		606 (100%)	564 (93%)	42 (6.9%)	
>7		190 (100%)	177 (93%)	13 (6.8%)	
No. flock milk recording tests in the 22-23 season - categorized	856				>0.9[3]
None		528 (100%)	492 (93%)	36 (6.8%)	
One or more		328 (100%)	306 (93%)	22 (6.7%)	
Pulsation frequency - categorized	737				>0.9[3]
<130/min		289 (100%)	269 (93%)	20 (6.9%)	
≥130/min		448 (100%)	418 (93%)	30 (6.7%)	
System pressure - categorized	812				0.5[3]
<38 kPa		195 (100%)	184 (94%)	11 (5.6%)	
≥38kPa		617 (100%)	574 (93%)	43 (7.0%)	
No. seasons the farm has operated (including 22/23 season) - categorized	856				**0.2[3]**
2-3		476 (100%)	439 (92%)	37 (7.8%)	
4-25		380 (100%)	359 (94%)	21 (5.5%)	

[1]Values < 0.2 are in bold.

[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); and group 3 = severe hyperkeratosis (both teat ends ≥ 3).

[3]Pearson's Chi-squared test

[4]Fisher's exact test

Supplemental Table 5: Univariable logistic regression models of potential risk factors for subclinical mastitis, in a study of subclinical mastitis of randomly selected ewes (n=894) on 20 commercial dairy sheep farms in New Zealand.

Variable	N	OR (95% CI)	p-value
Visit	856		**0.11**
Visit 1		—	
Visit 2		0.74 (0.40-1.37)	
Visit 3		0.48 (0.23-0.96)	
Farm	856		**0.077**
A		—	
B		1.23 (0.05-31.6)	
C		7.71 (1.18-151)	
D		3.95 (0.49-81.5)	
E		1.32 (0.05-34.0)	
F		4.26 (0.52-88.0)	
G		2.79 (0.35-57.4)	
H		5.14 (0.73-103)	
I		3.86 (0.35-85.1)	
J		4.05 (0.50-83.5)	
K		0.00 (0.00-33,442)	
L		11.8 (1.98-226)	
M		2.51 (0.23-55.1)	
N		8.64 (1.51-163)	
O		2.45 (0.23-53.8)	
P		7.30 (1.12-143)	
Q		1.86 (0.07-48.3)	
R		5.40 (0.76-108)	
S		1.86 (0.07-48.3)	
T		3.86 (0.35-85.1)	
Age (yr)	633	1.14 (0.92-1.41)	0.22
Body condition score	853	1.16 (0.70-1.91)	0.56
Days in milk	464	1.00 (0.99-1.00)	0.70
No. lambs born	398	1.31 (0.77-2.20)	0.32
Udder depth score[1]	856		0.29
2		—	
3		0.25 (0.03-5.18)	
4		0.42 (0.07-8.06)	
5		0.23 (0.03-4.71)	
Udder suspension score	856		0.89
1		—	
2		0.89 (0.28-3.94)	
3		0.78 (0.25-3.42)	
4		0.80 (0.24-3.67)	
5		0.49 (0.09-2.74)	

Variable	N	OR (95% CI)	p-value
Udder separation score	854		0.36
1		—	
2		0.54 (0.17-2.41)	
3		0.51 (0.16-2.26)	
4		0.27 (0.06-1.34)	
5		0.26 (0.01-2.17)	
Teat placement score[1]	856		0.70
2		—	
3		1.83 (0.63-7.74)	
4		1.70 (0.56-7.38)	
5		1.23 (0.22-6.88)	
Udder symmetry	854		**0.002**
Yes		—	
No		2.37 (1.38-4.12)	
Udder inflammation	851	1.05 (0.06-5.43)	0.96
Udder lesions	847	0.92 (0.15-3.16)	0.91
Udder palpation	856		0.45
Normal		—	
Lump		1.86 (0.29-6.83)	
Teat inflammation[2]	852	Not evaluated	
Teat lesions	853	0.80 (0.04-4.02)	0.83
Teat palpation	856		0.37
Normal		—	
Abnormal		0.55 (0.09-1.83)	
Teat end hyperkeratosis[3]	846		**0.061**
Group 1		—	
Group 2		7.11 (0.97-37.3)	
Group 3		7.11 (0.33-75.3)	
Ewe median teat length (mm)	852	1.00 (0.94-1.06)	0.91
Ewe median teat width (mm)	852	1.03 (0.94-1.13)	0.50
Age (yr categorized)	856		0.27
Hogget		—	
2-tooth		2.08 (0.84-5.62)	
MA		1.69 (0.79-4.22)	
Farm area (Ha)	856	1.00 (0.99-1.00)	0.27
Peak number of ewes milked	856	1.00 (1.00-1.00)	0.75
Total number of staff - early lactation	856	0.95 (0.81-1.09)	0.49
Total number of staff - mid-late lactation	856	0.93 (0.78-1.08)	0.36
Number of milking staff - early lactation	856	0.87 (0.57-1.32)	0.52
Number of milking staff - mid-late lactation	856	0.68 (0.42-1.06)	**0.091**
Lambing to lamb removal interval (days)	796	1.00 (0.98-1.01)	0.73
Mean season milk production per ewe (L)	604	1.00 (0.99-1.01)	0.85
Use of gloves during milking	856		0.68

Variable	N	OR (95% CI)	p-value
Compulsory/all		—	
Optional/some		0.93 (0.54-1.63)	
Not worn		0.45 (0.02-2.24)	
Teat spray use	856		**0.12**
All season		—	
Part season		1.80 (1.01-3.35)	
Not used		1.77 (0.62-4.43)	
Teat spray type	782		0.58
Chlorhexidine		—	
Iodine		0.82 (0.38-1.61)	
Milking frequency	856		**0.11**
Twice daily		—	
Twice daily for part of the season then once daily		0.74 (0.42-1.31)	
Once daily		0.42 (0.17-0.96)	
Automatic cup removers	856	0.70 (0.38-1.24)	0.23
Inline milk meters	856	1.28 (0.68-2.29)	0.43
Pulsation frequency	737	1.00 (0.99-1.02)	0.64
System pressure (kPa)	812	1.11 (1.00-1.25)	**0.052**
Type of flow line (high/low)	856		**0.013**
High		—	
Low		0.50 (0.27-0.87)	
Automatic feeding system	856	1.17 (0.60-2.49)	0.66
No. seasons the farm has operated (including 22/23 season)	856	0.98 (0.90-1.04)	0.59
No. flock milk recording tests in the 22-23 season	856	1.05 (0.91-1.20)	0.48
Udder depth score - categorized	856		0.44
1-2		—	
3		0.25 (0.03-5.18)	
4-5		0.38 (0.06-7.33)	
Udder suspension score - categorized	856		0.78
1-2		—	
3		0.87 (0.46-1.62)	
4-5		0.79 (0.39-1.56)	
Udder separation score - categorized	854		**0.17**
1-2		—	
3		0.89 (0.50-1.59)	
4-5		0.46 (0.18-1.05)	
Teat placement score - categorized	856		0.57
1-2		—	
3		1.83 (0.63-7.74)	
4-5		1.62 (0.55-6.97)	
Udder palpation - categorized	856		0.45
Normal		—	
Abnormal		1.86 (0.29-6.83)	

Variable	N	OR (95% CI)	p-value
Teat end hyperkeratosis - categorized[3]	846		**0.018**
Group 1		—	
Group 2 or 3		7.11 (1.47-27.7)	
Body condition score - categorized	853		0.70
1-2		—	
2.5-4		0.90 (0.53-1.55)	
No. lambs born - categorized	398		0.39
1		—	
2		1.81 (0.77-4.75)	
≥3		1.73 (0.49-5.67)	
Lambing to lamb removal interval (days) - categorized	796		0.97
1-7		—	
>7		0.99 (0.50-1.83)	
No. flock milk recording tests in the 22-23 season - categorized	856		0.95
None		—	
One or more		0.98 (0.56-1.69)	
Pulsation frequency - categorized	737		0.91
<130/min		—	
≥130/min		0.97 (0.54-1.76)	
System pressure - categorized	812		0.51
<38 kPa		—	
≥38kPa		1.25 (0.66-2.60)	
No. seasons the farm has operated (including 22/23 season) - categorized	856		**0.19**
2-3		—	
4-25		0.69 (0.39-1.20)	

[1]No ewes had a score of 1.

[2]No teat inflammation was diagnosed among ewes with subclinical mastitis.

[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); and group 3 = severe hyperkeratosis (both teat ends ≥ 3).

Abbreviations: CI = Confidence Interval, OR = Odds Ratio, OR = odds ratio, CI = confidence interval., Values <0.2 are in bold.

6 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

Chapter 6 is the second of two companion articles on subclinical mastitis published in the Journal of Dairy Science. Chapter 5 (the first of the companion articles) set the scene by defining subclinical mastitis and reporting its prevalence and identifying risk factors. It established that, while the bacterial causes in New Zealand ewes are similar to those of ewes overseas, the prevalence is significantly lower. That is good news for our farmers and the New Zealand industry. Only two risk factors were identified: teat end hyperkeratosis and udder asymmetry, and the prevalence decreased through the lactation.

Chapter 6 extends Chapter 5 by exploring diagnostic tests for subclinical mastitis and whether it is linked to elevated aerobic plate count (a measure of overall bacterial load in milk). The information presented here equips farmers and veterinarians with knowledge on the strengths and weaknesses of somatic cell count and the rapid mastitis test and clarifies how they should be used.

These two chapters were published as companion articles in the Journal of Dairy Science and are in press at the time this thesis was written. Chapter 6 can be found at https://doi.org/10.3168/jds.2025-27076.

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Greg Chambers
Name and title of main supervisor:	Professor Richard Laven
In which chapter is the manuscript/published work?	6 - Subclinical mastitis part 2

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

The student obtained the funding, designed the protocol, collated and analysed the data, and lead the manuscript writing and responses to peer reviewers.

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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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6.1 Abstract

Our objectives were to, in grazing dairy ewes, 1) describe SCC, California mastitis test (CMT) score, and ewe-level milk aerobic plate count (APC), 2) explore the relationship between CMT and SCC, 3) identify risk factors for elevated APC, and 4) find the optimal SCC threshold for diagnosis of IMI. Gland-level milk samples were collected from approximately 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, and 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 somatic cell count was 169,039 (95% CI: 153,921-185,641) cells/mL, varying between farms and decreasing across visits. A CMT score ≥1 in 1 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 *S. aureus* (0.6% of glands). A SCC threshold of approximately 400,000 cells/mL had the greatest Youden's index for diagnosing IMI using a single SCC measurement. APC was below the limit of quantification (1 x 10^3 CFU/mL) in 78.0% of ewes, <100 x 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.

Keywords: sheep, milk quality, mastitis, aerobic plate count

6.2 Introduction

Compared to 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. There were an estimated 30,000 ewes being milked on approximately 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.* 2016), 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.

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), and 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 elevated somatic cell count (SCC) (Fthenakis 1994; Lysitsas *et al.* 2024) and/or California mastitis test (CMT) (Las Heras *et al.* 1999) and/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 (SCC) 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 California mastitis test (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 SCCs. 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 x 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 the 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, as has been shown for dairy cattle (Zadoks *et al*. 2004). However, this hypothesis has not been formally tested, and it is possible that changes in APC were coincidental. While 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 (Gonzalez-Rodriguez *et al.* 1995; Berthelot *et al.* 2006; Lafi 2006; Riggio *et al.* 2013), but there is some variation in the thresholds and in the methods used to determine them and the study populations, and large-scale studies performed on grazing systems are lacking.

Our objectives were to systematically describe individual ewe SCC, CMT, APC, milk bacteriology results, determine the correlation between CMT and SCC, identify risk factors for elevated ewe milk APC, and 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 Europe.

6.3 Materials and Methods

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

6.3.1 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 (Anonymous 2020), and given the rapid expansion, it was estimated that at the start of the 2022/2023 milking season there were approximately 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 (ICC) of 0.06 (Barkema *et al.* 1997), a sample size of 30 ewes per farm per visit (1,800 ewes in total across three visits) would have allowed a 26% prevalence to be estimated with a 95% confidence interval 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*. (2025b).

6.3.2 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.* 2025a). 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 three 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 - October 2022 (visit 1), November - 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).

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 hours after the morning milking to avoid prolonging milking time, except for 1 farm, where ewes were examined prior to 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 they were 1) under treatment or had

been treated within the previous 30 days for illness; 2) were diagnosed with clinical mastitis on the day of sampling (defined as visual or palpable udder changes with clots in the milk); 3) 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 and/or feces that the operator deemed it unlikely that milk samples would be uncontaminated. However, no ewes presented with any of these exclusion criteria.

6.3.3 Study procedures

6.3.3.1 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*. (2025a) and summarized in Chambers *et al*. (2025b).

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 approximately 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 while on farm, which was measured on a 5-point scale (negative, trace, 1, 2, 3) as described by Schalm *et al.* (1957). The remaining milk from each gland was then combined into a single composite sample, gently mixed, and then 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 hours 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).

6.3.3.2 Laboratory procedures

Somatic cell count and APC were measured at the ewe level and aerobic bacterial culture at the gland level. SCC was determined using a Combifoss 7 machine (Foss, Cambridge, New Zealand). APC was estimated by incubating samples at 30°C for 72 hours on milk plate count agar and counting the number of colonies to calculate the number of colony forming units (CFU) per mL of milk. Plates with no CFU 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 for individual ewe milk samples has been previously described (Chambers *et al.* 2024) and is summarized in Chambers *et al*. (2025b).

6.3.4 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, 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 below.

6.3.4.1 Somatic cell count and California mastitis test

Being heavily skewed, SCC was reported as raw and $\log_{10}$ SCC and categorized into "normal" ($<500 \times 10^3$ cells/ml), "intermediate" (between 500×10^3 and 1×10^6 cells/ml), and

"high" (>1 x 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^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 R-hat 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 two 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 two 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 two 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 tau-a. The Wilson method was also used to generate confidence intervals for the proportions of glands in each CMT score.

6.3.4.2 Aerobic plate count

The minimum limit of quantification for APC was 1 x 10^3 CFU/mL, below which results were reported as <1 x 10^3 CFU/mL, and the maximum limit of quantification was 3 x 10^6 CFU/mL, above which results were reported as 3 x 10^6 CFU/mL. APC results of "QC" (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 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 x 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; 4) ≥100,000 CFU/mL. We also dichotomized APC into "low" (<100 x 10^3 CFU/mL) and "high" (≥100 x 10^3 CFU/mL) because 100 x 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 APCs of 100 x 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.

6.3.4.3 Bacterial culture

Bacterial culture results were descriptively reported at the gland level in Chambers *et al*. (2025b). Results were collapsed to the ewe level by categorizing ewes into having IMIs due to 1) *S. 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 two glands returned different results (from the set of NAS, *S. aureus*, and other) and neither gland was infected with *S. aureus* or contaminated, and 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 one 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.

6.3.4.4 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 and/or SCC >500 x 10^3 cells/ml (Fragkou *et al.* 2014). A bacteriologically positive milk sample with no increased CMT score (<1) or SCC <500 x 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.

6.3.4.5 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 and 2-3), dichotomized CMT score (0-T and 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, since <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 leave-one-out cross-validation (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. 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 versus 1,000-9,999, 10,000-99,999, and ≥100,000,

<1,000 and 1,000-9,999 versus 10,000-99,999, and ≥100,000, and <1,000, 1,000-9,999 and 10,000-99,999 versus ≥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, i.e., uniform distributions for fixed effects and Student *t* distributions (3 degrees of freedom, mean = 0, scale = 2.5) for intercepts and SDs. If the posterior means and standard deviations changed by at most 20% and the ELPD comparison was not significant, the priors were accepted.

6.3.4.6 Somatic cell count 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 if there were 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 to the no growth category. Contrasts for categorized culture results were adjusted for multiple comparisons with the Šidák method (Sidak 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 x 10^3, 500 x 10^3, 750 x 10^3, and 1 x 10^6 cells/ml.

6.4 Results

6.4.1 Enrolment and data

Across the three visits, 893 observations were made on 882 unique ewes. Eleven ewes were examined at two visits by chance; however, three of these lacked ear tags and may, in fact, represent six different untagged ewes. No ewes were excluded at the selection or data analysis stages. Visits 1-3 were conducted on 24 August to 6 October 2022, 7 November to 22 December 2022, and 25 January to 16 March 2023 respectively. Outside of the first three 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 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 supplementary materials of Chambers *et al*. (2025a), and the reasons for missing data are summarized in the supplementary materials of the present article. Farm and ewe information are summarized in Chambers *et al*. (2025b).

6.4.2 Somatic cell count

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 - 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.

There were 748, 53, and 89 samples with 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 - 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 to the variance between farms, as indicated by the large spread within farms and minimal differences in median values across farms (Figure 1).

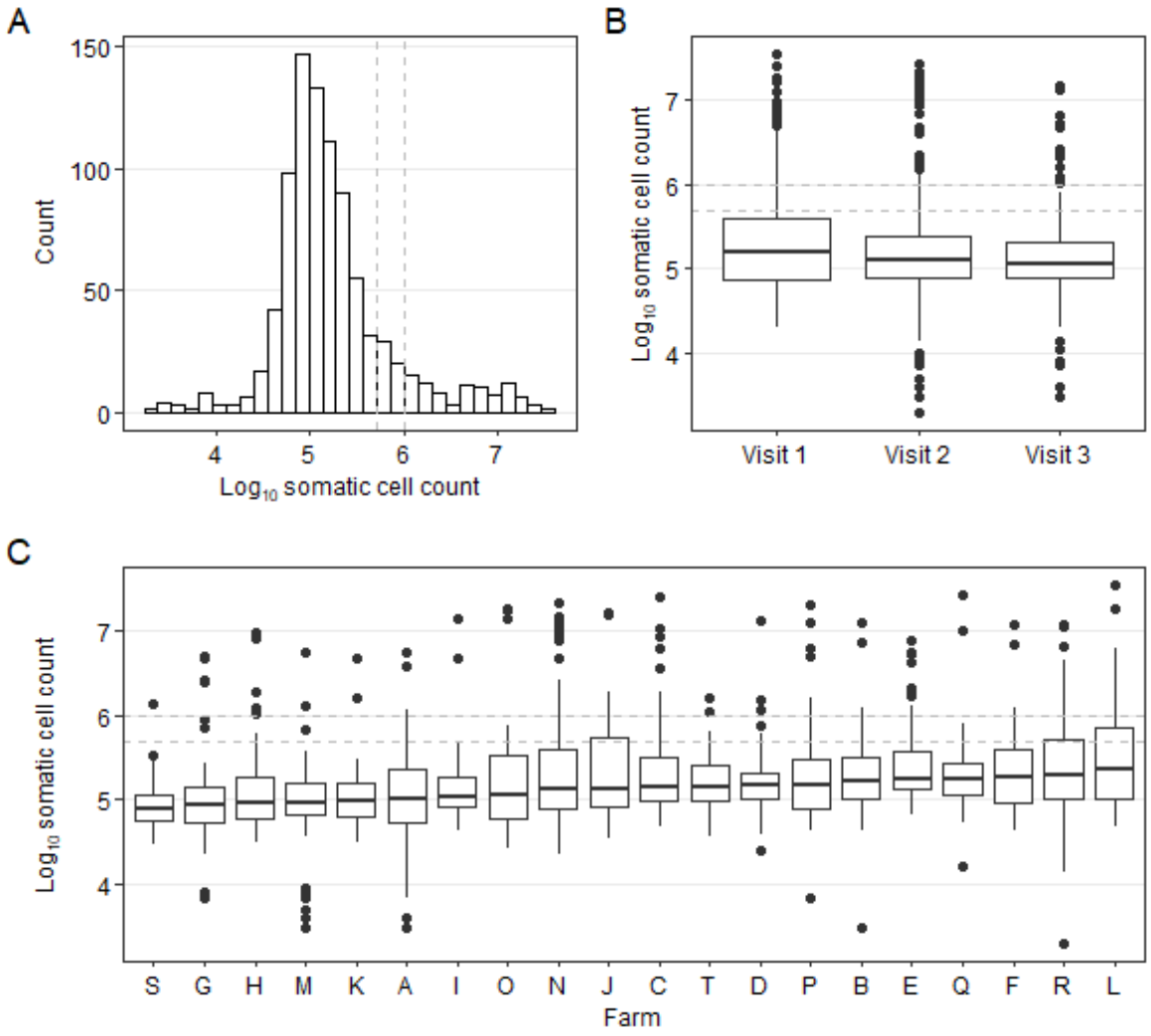

Figure 1: Distributions of log_{10} somatic cell count (A) overall, (B) by visit, and (C) by farm (ordered on mean log somatic cell count), in a study of milk quality of randomly selected ewes (n=894) on 20 commercial dairy sheep farms in New Zealand. Boxes extend from the first to the third quartiles, 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 interquartile range from the third quartile. Lower whiskers extend from the first quartile to the lowest value that is no more than 1.5 times the interquartile range from the first quartile. Data beyond the end of the whiskers are deemed outliers and are plotted individually. Dashed lines indicate somatic cell counts of 500 x 10^3 and 1 x 10^6 cells/mL.

6.4.3 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 only (i.e., the other gland had a score <1).

6.4.4 Agreement between somatic cell count 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 x 10^6 and 1 x 10^6 cells/ml, are shown for each CMT score in Table 1.

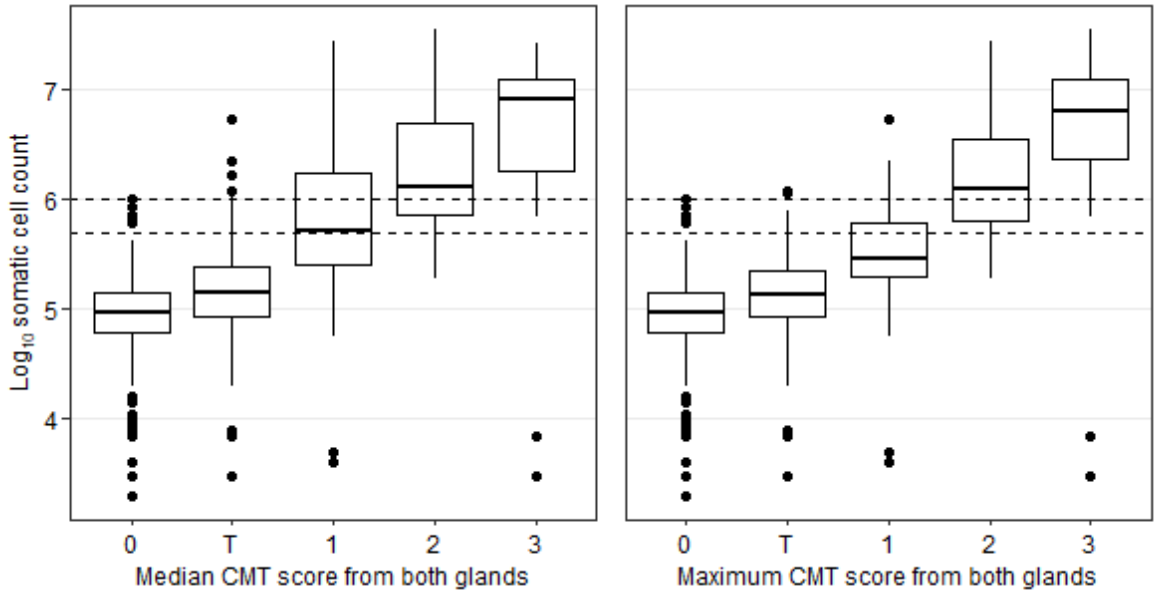

Figure 2: Distribution of $\log_{10}$ somatic cell count for each California mastitis test (CMT) score when A) ewes were assigned the median score of the two glands (rounded up to the nearest score), or B) the maximum score, in a study of udder health of randomly selected ewes (n=894) on 20 commercial dairy sheep farms in New Zealand. CMT was scored using a scale of 0, trace, 1, 2, and 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 interquartile range, and outliers are shown as individual dots. Dashed lines indicate somatic cell counts of 500 x 10^3 and 1 x 10^6 cells/mL.

Table 1: Distribution of somatic cell count (SCC) for each ewe California mastitis test (CMT) score when ewes were assigned the median score of the two 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. CMT was scored on a scale of 0, trace, 1, 2, and 3. CMT and SCC data were available for 870/893 ewes.

Method	N	Median (range) SCC	SCC >0.5 x 10^6 cells/ml[1]	SCC >1 x 10^6 cells/ml[1]
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]n (%)

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

6.4.5 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 x 10^3 - 3 x 10^6 CFU/mL. Overall, 682/874 (78%) milk samples had an 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. There were 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 APCs ≥100,000 CFU/ml recorded on 9/20 farms (Figure 3).

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.

	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

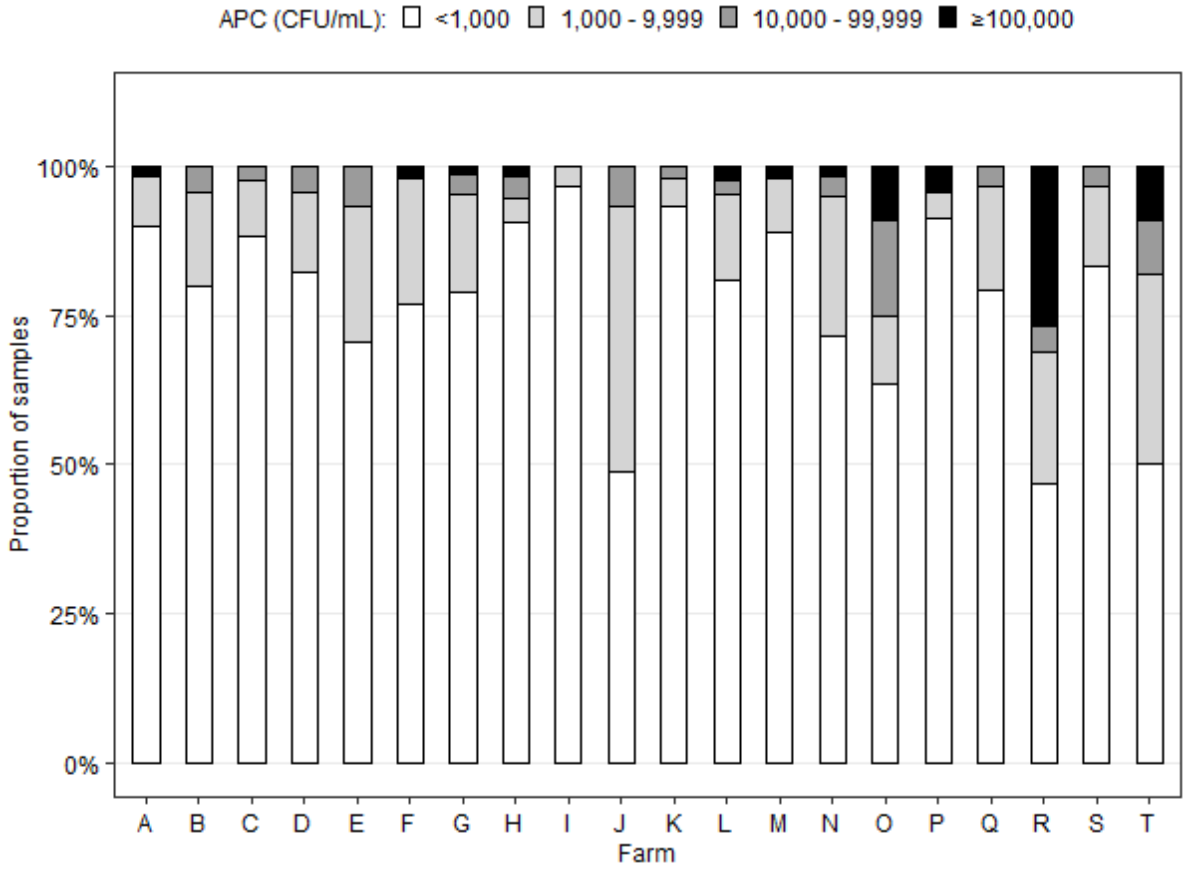

Figure 3: Distribution of aerobic plate count (APC) of ewe milk samples on each farm in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand.

6.4.6 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 supplementary materials.

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					
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%)
S. 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

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
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					
Visit 1	285 (33%)	213 (31%)	54 (41%)	10 (31%)	8 (30%)
Visit 2	294 (34%)	210 (31%)	49 (37%)	19 (59%)	16 (59%)
Visit 3	295 (34%)	259 (38%)	30 (23%)	3 (9.4%)	3 (11%)

All models except the model for udder symmetry significantly improved the fit of the data compared to 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/negative), bacterial class, median CMT, $\log_{10}$ SCC, and visit. Ewes with CMT scores 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 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 ewes with subclinical mastitis had 10.77 times (95% CI = 6.17-18.77) higher odds of being in a higher APC category than ewes without subclinical mastitis. Predicted probabilities of each APC category are shown in Figure 4. The models met all the diagnostic criteria.

Table 4: Odds ratios and 95% credible intervals 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, and ≥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.

Risk factor model	Fixed effect	Odds ratio (95% CI)	ELPD[1]	Model significance[2]
Dichotomized CMT	0-1	(Reference)	-536.6925	*
	2-3	6.7 (4.36-10.22)		
Maximum CMT	0	(Reference)	-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	(Reference)	-538.9855	*
	Positive	10.77 (6.17-18.77)		
Bacterial culture (positive/negative)	Negative	(Reference)	-542.5518	*
	Positive	7.32 (4.46-11.77)		
Bacterial culture (class)	No growth	(Reference)	-543.0292	*
	S. 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	(Reference)	-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	Log_{10} SCC	2.37 (1.82-3.11)		*
Visit	Visit 1	(Reference)	-555.9055	*
	Visit 2	1.31 (0.89-1.97)		
	Visit 3	0.35 (0.22-0.58)		
Udder symmetry	Symmetrical	(Reference)	-572.7749	
	Asymmetrical	1.32 (0.94-1.82)		

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

Risk factor model	Fixed effect	Odds ratio (95% CI)	ELPD[1]	Model significance[2]

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

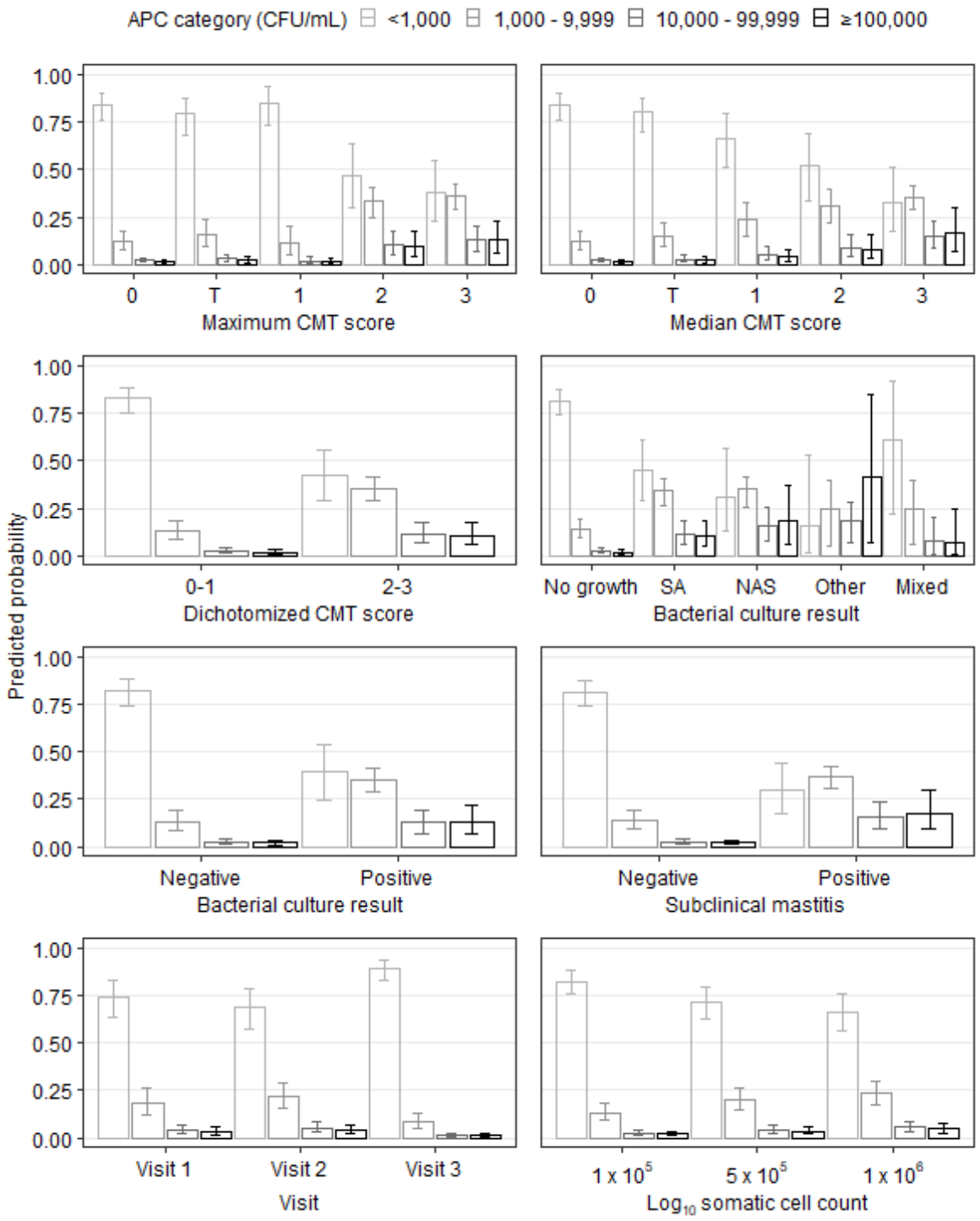

Figure 4: Distribution of aerobic plate count (APC) of ewe milk samples on each farm in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand, by California mastitis test (CMT) score. CMT was scored using a scale of 0, trace, 1, 2, and 3. SA = *Staphylococcus aureus*, NAS = non-aureus staphylococci.

6.4.7 Somatic cell count thresholds for the 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 to no growth.

Table 5: Mean (95% CI) log_{10} somatic cell count (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[1]	N (%)	Mean (95% CI)[2]	P value[3]
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
S. aureus	5 (0.6)	6.26 (5.73 - 6.79)	<0.001

[1]NAS = non-aureus staphylococci.

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

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

The diagnostic performance of SCC thresholds of 250,000, 500,000, 750,000 and 1,000,000 cells/mL, and 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, while *S. aureus* had a higher optimal threshold at 799,834 cells/mL. The areas under the ROC curves 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.

Table 6: Optimal somatic cell count (SCC) thresholds for diagnosing intramammary infection (IMI) in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand.

IMI	SCC threshold (cells/mL)[1]	Se[2]	Sp[3]	PPV[4]	NPV[5]	YI[6]
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
S. 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

[2]Sensitivity

[3]Specificity

[4]Positive predictive value

[5]Negative predictive value

[6]Youden's index

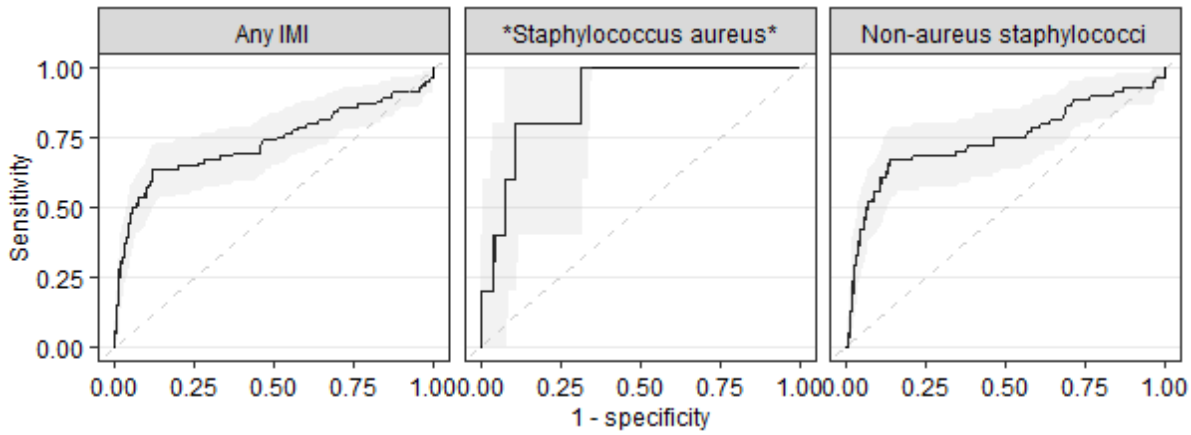

Figure 5: Receiver operator characteristic curves of the performance of somatic cell count at diagnosing intramammary infection in a study of udder health of randomly selected ewes (n=893) on 20 commercial dairy sheep farms in New Zealand. The shaded gray area represents the 95% CI.

6.5 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 to 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.

There is an anecdotal perception that ewe SCCs are generally much higher than cow SCCs, but this study did not support that perception. The geometric mean ewe SCC was 169 x 10^3 cells/mL, which was the same as the mean dairy cow milk-recording SCC reported for the same (2022-2023) season (DairyNZ and Livestock Improvement Corporation 2023). We did find a substantial right tail for SCC among the ewes in our study. In fact, there were more ewes in the "high" (>1 x 10^6 cells/ml) category than the "intermediate" category (between 500 x 10^3 and 1 x 10^6 cells/ml) (10.0 and 6.0% respectively), underscoring the value of monitoring ewe SCC by routine SCC measurement and/or screening with the CMT.

The SCCs were low compared to those described by McDougall *et al*. (2001), who reported a geometric mean of 539 x 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 days postpartum, when they may have had higher SCCs, as was seen in the present study where there was a higher mean SCC at the first visit compared to later visits. In contrast, Ariznabarretta *et al*. (2002) found a geometric mean of 72,444 (log10 4.86) cells/mL and Gonzalo *et al*. (2002) found a geometric mean 95,499 (log10 4.98) cells/mL using similar methodology among bacteriologically negative dairy ewes in Spain. These SCCs were approximately half the mean $\log_{10}$ of 5.16 observed in culture-negative ewes in the present study (Table 5). It is unclear why there was such a large difference between the studies, but it may reflect different management systems. The flocks in the study of Ariznabarretta *et al*. (2002) were not involved in any mastitis control programs, while 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.

Freezing of milk samples prior to culture may have influenced bacterial recovery. While some studies have shown reduced recovery of certain pathogens, particularly in low CFU samples and for Gram-negative bacteria, others have reported increased or unchanged isolation rates depending on the organism. 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 weeks. The proportion of samples that were bacteriologically positive declined by up to 50% with time across all pathogens, and the lower the CFU count of the sample, the more its viability was impacted by freezing. However, for culture-negative samples on Day 0, freezing increased the isolation rate for *S. aureus*. Sanchez *et al*. (2003) demonstrated that freezing milk samples from goats infected with subclinical mastitis at −20°C or −80°C up to 730 days increased the

CFU count of coagulase-negative staphylococci and reduced the CFU 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 weeks reduced the number of samples that had cultures of *E. 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 freezing reduces the viability of some bacterial species in milk, especially in low CFU count samples, 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 SCCs 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 hours after milking. The samples therefore reflect cisternal milk, not alveolar milk. However, McKusick *et al*. (2002) demonstrated that there was no difference between cisternal and alveolar milk in SCC for samples collected from dairy ewes within 12 hours of milking.

Substantial between-farm differences in $\log_{10}$ SCC were confirmed, with an approximately 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 to the variance between farms in our dataset (Figure 1), meaning there are 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 only explained 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, since we did not sample the same ewes at each visit, or a natural physiological decrease in SCC along the lactation. It aligns with the decline in subclinical mastitis prevalence reported in Chambers *et al*. (2025b). 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 x 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 x 10^6 or 1 x 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, there was wide variation in SCC at each CMT score and hence the Kendall's tau 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 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 APCs below the limit of quantification, which supports a generally robust sample collection and handling procedure. Only 3.1% of ewes had APCs >100 x 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 x 10^3 CFU/mL. The reasons for these differences are not clear, but may be due to differences in teat hygiene, intramammary infection, or contamination from the teat surface or environment during sampling. 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, there is evidence 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.* 2025b). This may be because udder asymmetry is a chronic change and, while it may be associated with SCC, ewes with asymmetric udders are not necessarily shedding large numbers of bacteria. *S. aureus* had a weaker association with elevated APC than NAS and other or mixed IMIs, suggesting that *S. aureus* IMIs result in less bacterial shedding into the milk. The category “other” had a relatively strong association with elevated APC. This category included *Strep. uberis* and *E. coli*, both of which have been linked to elevated bulk

milk APC in bovine dairy operations (Zadoks *et al*., 2004). The odds of elevated APCs were lower at visit 3, which may reflect selective removal of ewes or the drier late summer weather.

While 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, the diagnostic performance of dichotomised CMT for detecting APC $\geq 100 \times 10^3$ was limited. The positive predictive value was low: only 11/109 (10.1%) ewes with CMT scores of 2 or 3 had APC $\geq 100 \times 10^3$. The negative predictive value was high, with 730/746 (97.9%) ewes with CMT scores of 0, trace, or 1 having APC $< 100 \times 10^3$, but this was partly a consequence of the low prevalence of elevated APC. Dichotomised CMT identified only 11/27 (40.7%) ewes with APC $\geq 100 \times 10^3$, meaning that most high-APC ewes would not have been detected using CMT alone. Therefore, while ewes with a high dichotomized CMT score have much higher odds of elevated APCs, 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 APCs 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.

Ewes with IMI due to any pathogen, NAS, or *S. aureus* had higher mean SCCs than ewes with no IMI. Of the three pathogen groups, *S. aureus* had the highest SCC. 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. However, pathogen-specific differences in SCC response are plausible, with Schepers *et al*. (1997) reporting different magnitudes of SCC elevation among pathogens in 22,467 milk samples from 544 dairy cows.

Somatic cell count showed good overall discriminatory ability based on ROC AUCs, but application depends on the chosen threshold. Optimal SCC thresholds of approximately 400,000 cells/mL were identified for IMI due to any pathogen and NAS, and approximately 800,000 cells/mL for *S. aureus*. At those thresholds, the sensitivities for detecting IMIs 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 impact of freezing samples prior to culture. In contrast, Riggio *et al*. (2013) calculated similar AUCs 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, an SCC of 400,000 cell/mL would seem to be 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, since a negative test result is more likely to mean a true negative when the prevalence is low. The specificities for all three pathogen groups were >0.8, but while specificity is less of a concern because positive ewes (ewes with SCCs 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 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 SCCs in the presence of IMIs due to the lack of dilution by the contralateral gland. However, we chose to use a ewe-level approach since that is how routine flock recording is performed. Knuth *et al*. (2019) found that Youden's index was maximized at a SCC threshold of 1,375 x 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. Gonzalez-Rodriguez *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 equalled 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/transiently infected, and infected/persistently infected. To be diagnosed as infected, ewes needed to have >1 SCC >1 x 10^6 cells/mL. This approach would require adaptation prior to applying it to other systems such as in New Zealand where milk recording, when performed, may only occur 2-4 times in a lactation.

When viewed alongside international studies, our findings highlight both commonalities and differences between New Zealand grazing systems and established international dairy sheep 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 years. 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 intramammary infections 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 similar proportion of ewes with CMT score <1 as Fthenakis (1995). The SCC threshold that optimized diagnostic accuracy for intramammary infection was lower than that of Knuth *et al*. (2019) in the USA and Riggio *et al*. (2013) in Italy, but higher than that of Gonzalez-Rodriguez *et al*. (1995) in Spain. However, these thresholds are not directly comparable because they depend on the study population, infection prevalence, pathogen distribution, refence definition, and the criteria used to select the threshold, such as optimising for sensitivity, specificity, or their balance. 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), showing that ewes with subclinical mastitis tend to have higher APC under grazing conditions. This supports the biological expectation that intramammary infection contributes to bacterial load in milk, although APC may also be influenced by other sources such as teat surface contamination and sample handling. These comparisons emphasize that while 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.

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 dataset, identify areas for improvement, inform future studies, and compare future studies against. 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 like in New Zealand, and including economic considerations in SCC threshold selection.

6.6 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 that most of its 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. A 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, RMT, 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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6.8 Notes

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Nonstandard abbreviations used: APC = aerobic plate count; AUC = area under the curve; CMT = California Mastitis Test; 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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6.10 Supplementary information

Supplementary Table 1: Number of observations missing raw data, and the reasons, in a study of udder health of randomly selected ewes (n=894) on 20 commercial dairy sheep farms in New Zealand.

Variable	N missing	Reason
Ewe information		
Age	234	Unknown by farmer (n=234)
BCS	3	Not recorded (n=3)
Breed	302	Unknown by farmer (n=302)
Recorded no. lambs born	821	Unknown by farmer (n=821)
Recorded no. lambs at pregnancy scanning	484	Unknown by farmer (n=484)
Recorded lambing date	414	Unknown by farmer (n=414)
Recorded first milking date	454	Unknown by farmer (n=454)
Milk information		
Somatic cell count	3	Mislabelled samples (n=2), no sample (n=1)
CMT - left gland	16	Atrophic/non-lactating gland (n=8), result not recorded (n=7), no sample taken (n=1)
CMT - right gland	13	Atrophic/non-lactating gland (n=4), result not recorded (n=7), no sample taken (n=2)
Aerobic plate count	19	Quality control (n=10), no sample (n=6), mislabelled (n=2), spreader (n=1)
Microbiology result - left gland	12	Missing sample (n=9), mislabelled (n=2), no sample (n=1)
Microbiology result - right gland	11	Missing sample (n=8), mislabelled (n=2), no sample (n=1)

Supplementary table 2: 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, and ≥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.

Risk factor model	Parameter	Odds ratio (95% CI)	ELPD[1]	Model significance[2]
Dichotomized CMT	Intercept 1	1.59 (1.12-2.07)	-536.6925	*
	Intercept 2	3.17 (2.65-3.72)		
	Intercept 3	4.07 (3.46-4.66)		
	0-1	(Reference)		
	2-3	1.9 (1.47-2.32)		
	Random intercept	0.93 (0.53-1.31)		
Maximum CMT	Intercept 1	1.68 (1.2-2.23)	-537.3385	*
	Intercept 2	3.27 (2.68-3.81)		
	Intercept 3	4.17 (3.59-4.84)		
	0	(Reference)		
	T	0.32 (-0.13-0.79)		
	1	-0.14 (-0.86-0.56)		
	2	1.81 (1.18-2.39)		
	3	2.18 (1.56-2.82)		
	Random intercept	0.98 (0.61-1.46)		
Subclinical mastitis	Intercept 1	1.5 (1.08-1.96)	-538.9855	*
	Intercept 2	3.07 (2.58-3.56)		
	Intercept 3	3.95 (3.38-4.49)		
	Negative	(Reference)		
	Positive	2.38 (1.82-2.93)		
	Random intercept	0.95 (0.57-1.37)		
Bacterial culture	Intercept 1	1.55 (1.05-2.01)		
	Intercept 2	3.1 (2.56-3.61)		
	Intercept 3	3.97 (3.4-4.57)		
	Negative	(Reference)		
	Positive	1.99 (1.49-2.47)		
	Random intercept	1 (0.6-1.43)		
Ewe class	Intercept 1	1.52 (1.02-1.96)		
	Intercept 2	3.07 (2.55-3.6)		
	Intercept 3	3.98 (3.39-4.58)		
	No growth	(Reference)		
	Staphylococcus aureus	0.99 (-0.86-2.84)		
	NAS	1.73 (1.17-2.33)		
	Other	2.36 (1.35-3.34)		

Risk factor model	Parameter	Odds ratio (95% CI)	ELPD[1]	Model significance[2]
	Mixed	3.55 (1.46-5.58)		
	Random intercept	0.98 (0.57-1.38)		
Median CMT	Intercept 1	1.68 (1.18-2.21)	-546.3494	*
	Intercept 2	3.24 (2.69-3.8)		
	Intercept 3	4.12 (3.54-4.77)		
	0	(Reference)		
	T	0.26 (-0.17-0.68)		
	1	0.98 (0.46-1.55)		
	2	1.61 (1-2.32)		
	3	2.43 (1.7-3.21)		
	Random intercept	0.95 (0.57-1.37)		
Log_{10} SCC	Intercept 1	5.87 (4.39-7.34)		*
	Intercept 2	7.38 (5.75-8.81)		
	Intercept 3	8.25 (6.65-9.81)		
	Log_{10} SCC	0.86 (0.6-1.13)		
	Random intercept	0.85 (0.51-1.25)		
Visit	Intercept 1	1.08 (0.56-1.62)	-555.9055	*
	Intercept 2	2.6 (2.09-3.23)		
	Intercept 3	3.48 (2.84-4.11)		
	Visit 1	(Reference)		
	Visit 2	0.27 (-0.12-0.68)		
	Visit 3	-1.04 (-1.52--0.55)		
	Random intercept	0.96 (0.6-1.37)		
Udder symmetry	Intercept 1	1.4 (0.95-1.86)	-572.7749	
	Intercept 2	2.85 (2.36-3.37)		
	Intercept 3	3.7 (3.13-4.26)		
	Symmetrical	(Reference)		
	Asymmetrical	0.28 (-0.07-0.6)		
	Random intercept	0.86 (0.49-1.22)		

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

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

7 BULK MILK SOMATIC CELL COUNT AND AEROBIC PLATE COUNT

This chapter turns from ewe-level analysis to farm-level analysis by exploring bulk milk. Commercial dairy farms that sell milk to a processor receive quantity and quality information for each consignment of milk. Action limits are applied by the Ministry for Primary Industries (MPI) to bulk milk that is destined for human consumption. These action limits are regulatory trigger points: results exceeding them require notification and corrective action procedures under the farm dairy's Risk Management Programme. As well as being used to check milk quality and food safety, bulk milk data are very useful for monitoring mastitis. Somatic cell count is used to indicate the prevalence of mastitis in a flock. High bulk milk somatic cell counts often trigger mastitis investigations by suppliers, so they can remove ewes with mastitis and lower the somatic cell count. Processors aim for low somatic cell counts for market access reasons but also because high somatic cell counts adversely affect the processing of milk.

Challenges with high bulk milk aerobic plate count (a global measure of the bacterial load of raw milk) were introduced in Chapter 6. A relationship was established between elevated ewe-level aerobic plate count and mastitis. In the present chapter, the relationship between mastitis (somatic cell count) and aerobic plate is explored at the bulk milk level. This has been done by other researchers overseas, but more robust statistical methods were used in the present article.

The aim of Chapter 7 was to provide baseline information on bulk milk somatic cell count and aerobic plate count, set targets based on the best-performing farms, and determine if aerobic plate count is associated with somatic cell count and thus mastitis. Knowing the

answers to these questions will help farmers manage high aerobic plate counts in their bulk milk.

Chapter 7 is currently in press in the New Zealand Journal of Agricultural Research at the time this thesis was written.

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Bulk milk somatic cell count and aerobic plate count on New Zealand dairy sheep farms

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7.1 Abstract

Our objectives were to describe bulk milk somatic cell count (SCC) and aerobic plate count (APC), and their association, on New Zealand dairy sheep farms. We retrospectively collated data from 907 bulk milk SCC and APC consignments from 16 farms over the 2022-2023 season. Bulk milk SCC had a geometric mean of 659,491 cells/mL, with farm geometric means ranging from 358,198 to 1,278,382 cells/mL. Aerobic plate count exceeded 100,000 CFU/mL in 200/896 (22.3%) consignments, peaked in August, and the farm-level proportion exceeding this threshold varied from 2.9% to 100%. The lowest quarter of farms for season mean $\log_{10}$ SCC had a geometric mean SCC of 370,383 cells/mL, while the lowest quarter for the proportion of consignments exceeding 100,000 CFU/mL had 11/230 (4.8%) consignments above this threshold. A doubling in SCC was associated with approximately double the odds of APC exceeding 100,000 CFU/mL (OR = 2.09, 95% CI = 1.38-3.28), but high APC was not well predicted by SCC. We propose farm-level aspirational targets of 400,000 cells/mL and less than 5% of consignments exceeding 100,000 CFU/mL for SCC and APC, respectively. Routine milk recording and careful attention to mastitis detection, milking hygiene and plant maintenance may assist in achieving these targets.

Keywords: sheep; milk quality, somatic cell count, aerobic plate count.

7.2 Introduction

In New Zealand, all dairy farmers producing milk for human consumption are required by the Ministry for Primary Industries (MPI) to perform routine bulk milk testing of a suite of parameters, including somatic cell count (SCC) and aerobic plate count (APC), and the milk must be below certain thresholds, regardless of whether the farmer supplies a processor. The MPI action limits for SCC and APC of raw bulk sheep milk are 1,500,000 cells/mL and 100,000 CFU/mL, respectively (Anonymous 2025). These action limits are regulatory trigger points: results exceeding them require notification and corrective action procedures under the farm dairy's Risk Management Programme, rather than defining biological thresholds for mastitis or milk hygiene. Processors also typically set thresholds for bulk milk SCC and APC, which may be lower than the mandated thresholds, and producers may be penalised for exceeding these thresholds.

Bulk milk somatic cell count (BMSCC) is a widely used indicator of udder health and milk quality. It reflects the number of white blood cells in the milk, which increase in response to intramammary infection (Albenzio *et al.* 2019), and it has been shown to correspond to the prevalence of subclinical mastitis in dairy sheep (Fthenakis 2023). As such, BMSCC is useful not only for maintaining milk quality, but also monitoring mastitis within a flock and guiding intervention. Mastitis is a significant disease in dairy sheep. Clinical mastitis has both animal welfare and production impacts. Its incidence among New Zealand dairy ewes was estimated to be 2.3% per season, ranging from 0 to 6% at the farm level, with 25% of affected ewes exhibiting depression and/or fever and only 15% recovering without lasting problems (Chambers *et al.* 2024). Subclinical mastitis, defined as intramammary infection without visible signs of inflammation in the udder or milk (Fthenakis 1994; Las Heras *et al.* 1999; Lysitsas *et al.* 2024), is also 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), and the quality of products made from sheep milk such as cheese (Jaeggi *et al.* 2003). Among New Zealand dairy ewes, Chambers *et al*. (2025c) estimated the subclinical mastitis prevalence to be 6.4%, varying from 0 to 16.7% across farms, based on a case definition of a bacteriologically positive milk sample from one or both glands together with a rapid mastitis test score ≥1 and/or SCC >500 × 10^3 cells/mL.

Aerobic plate count assesses the bacterial load of the milk, which is typically associated with milking hygiene, plant sanitation and refrigeration effectiveness in dairy cow operations (Jayarao *et al.* 2004). However, anecdotal reports from New Zealand dairy sheep farmers indicate that high APC values (>100,000 CFU/mL) occur frequently and can be challenging to manage, even when common causes have been addressed. Reductions in APC have been observed after excluding ewes with high SCC or udder abnormalities from the milking herd. These observations suggest the hypothesis that mastitis can contribute to high bulk milk APC on dairy sheep farms, as has been shown for dairy cattle (Zadoks *et al*. 2004). Recent New Zealand research indicated that intramammary infection is a risk factor for elevated APC of individual ewe milk samples, and therefore potentially bulk milk as well (Chambers *et al.* 2025b), and a positive correlation between BMSCC and total bacterial count (TBC) has been demonstrated (de Garnica *et al.* 2013; Lianou *et al.* 2021). Furthermore, Lianou *et al*. (2021) showed that isolation of *Staphylococcus aureus* from the bulk tank milk was associated with elevated TBC. If BMSCC is positively associated with APC, control of SCC may contribute to managing APC, since they may have a causal relationship or share common risk factors, providing a useful tool to farmers.

Commercial dairy sheep farms have emerged recently in New Zealand and little is known about their typical BMSCC and APC profiles, how much they vary across time and between farms, and whether they are different to those of overseas flocks. This knowledge gap limits benchmarking of farm performance and impedes the establishment of quality targets.

Our objectives were thus to describe bulk milk APC and SCC on New Zealand dairy sheep farms and to determine their association.

7.3 Materials and Methods

7.3.1 Sample size

This study was part of a wider research program on mastitis in New Zealand dairy sheep, which was performed under the approval of the Massey University Animal Ethics Committee (application AEC 22/25). The sample size calculation was based on the primary objective of determining the prevalence of subclinical mastitis with acceptable precision, which was a function of the number of flocks enrolled and the number of ewes sampled per flock due to clustering of ewes within flocks. The total number of commercial dairy sheep farms in New Zealand at the time of study design was unknown. In 2019, there were 18 known farms (Anonymous 2020), and given the rapid expansion, it was estimated that at the start of the 2022/2023 milking season there were approximately 40 commercial farms. A target of 20 farms was set, corresponding to approximately 50% of New Zealand dairy sheep farms.

7.3.2 Study design, setting and participants

A retrospective, cross-sectional study was run on 16 commercial sheep dairy farms. The 20 farms in the wider research project were selected to represent diverse geographical locations and systems (Chambers *et al.* 2025a). Bulk milk data were available for 16 of these 20 farms (four farms processed their own milk and therefore did not have routinely collected bulk milk data). Data for the 2022-2023 season were obtained from the processors at the end of the 2022-2023 season, with the 12 farms in the Waikato region supplying two Waikato processors and the four farms in the Canterbury region supplying one Canterbury processor. The farms were located 0-200km from their respective processors (one processor was located on a supplying farm).

All 16 farms in this study lambed entirely in the spring. For hoggets, lambing start date varied between 25 July 2022 and 03 October 2022 (median 13 August), while for mixed-age ewes lambing start date varied between 02 July 2022 and 25 August 2022 (median 22 July). Milking commenced within 10 days of lambing on 14 farms, while two farms reared lambs on the ewes for at least five weeks. All ewes lambed outdoors except on two farms, where a subset of ewes lambed indoors (hoggets and ewes with three or more fetuses on one farm, and multiple bearing ewes during inclement weather on the other). The median peak number of ewes milked per farm was 838 ewes, ranging from 280 to 1,530 ewes. Ewes were dried off in batches, with some farms drying ewes off in several batches but others drying almost the whole flock off on one date. Milking ceased between mid-February and early May 2023, varying among farms.

7.3.3 Study procedures

Milk testing was performed by MilkTestNZ (Hamilton, New Zealand) for the Waikato farms and Eurofins (Christchurch, New Zealand) for the Canterbury farms. At both laboratories, SCC was determined using a Combifoss machine (Foss, Hillerød, Denmark) according to ISO 13366-2:2006 | IDF 148-2:2006. Aerobic plate count was estimated according to ISO 4833-1:2013 by incubating the samples at 30°C for 72 hours on milk plate count agar and counting the number of colonies to calculate the number of colony forming units (CFU) per mL of milk. The minimum and maximum detection limits were 2,500 and 2,500,000 CFU/mL, respectively, at MilkTestNZ. At Eurofins, the minimum and maximum detection limits were 1,000 and 3,000,000 CFU/mL, respectively.

7.3.4 Statistical analysis

Bulk milk data were provided as electronic spreadsheets (Excel, Microsoft, WA, USA) and imported into RStudio using R 4.5.1 for all analyses (R Core Team 2023). The raw data were

explored for completeness and spurious values. Both SCC and APC were $\log_{10}$ transformed due to their skew. Because APC was censored, and the laboratories applied different censoring thresholds, APC was not a truly continuous variable. We therefore dichotomised APC into ≤100,000 and >100,000 CFU/mL. This threshold was chosen because it is commonly applied by processors as a milk quality benchmark and is the acceptable limit set by MPI (Anonymous 2025). The Wilson method was used to calculate confidence intervals for proportions (Wilson 1927). For descriptive plots of $\log_{10}$ APC as a continuous variable, values at the minimum detection limit were set to half the detection limit, and values at the maximum detection limit were set to the detection limit. Descriptive statistics were calculated for SCC and APC overall, across time and for each farm. The farms were also ranked on mean $\log_{10}$ SCC and divided into quarters, with the mean and SD $\log_{10}$ SCC, and the geometric mean SCC and its 95% CI, calculated for the top and bottom quarters This was repeated separately for APC by ranking the farms on the proportion of consignments that exceeded an APC of 100,000 CFU/mL, and calculating the proportion for the top and bottom quarters, and its 95% CI. The geometric mean SCC and 95% CI were calculated with the formula:

$$\text{Geometric mean (95\% CI)} = 10^{\bar{x}_{\log_{10}\text{SCC}}}(10^{\bar{x}-1.96\cdot SE},\ 10^{\bar{x}+1.96\cdot SE})$$

We used four methods of increasing complexity to explore the relationship between SCC and APC. First, the relationship between SCC and APC was graphically explored with a scatter plot of $\log_{10}$ SCC versus $\log_{10}$ APC, overlaid with a linear regression line. To determine if the relationship varied across farms or across the milking season, scatter plots were constructed for each farm and for different periods of the season: August-September, October-mid-December, mid-December-January, and February-May. Second, we calculated Spearman's rank correlation coefficient to assess the linear relationship between $\log_{10}$ SCC and $\log_{10}$ APC. This treats each bulk milk consignment as independent and therefore does not account

for the correlation of repeated samples from the same farms. Third, the repeated measures correlation coefficient was calculated to assess the same relationship but accounting for correlation of repeated samples. This method uses ANCOVA to estimate the within-farm correlation of two paired variables (log_{10} SCC and log_{10} APC), assuming that all farms have the same slope but different intercepts (Bakdash and Marusich 2017). Confidence intervals for the Spearman's rank correlation and the repeated measures correlation coefficients were calculated using a bootstrap method with 1,000 iterations. Fourth, we constructed a regression model, using dichotomised APC (above or below a threshold of 100,000 CFU/mL) as the dependent variable, since APC was not a truly continuous variable due to censoring. We regarded this as the technically most robust approach because it does not assume the APC is truly continuous and accounts for correlation between repeated samples within farms.

Three regression modelling approaches were evaluated, all of which modelled the relationship between SCC and dichotomised APC (above or below a threshold of 100,000 CFU/mL) and included a random intercept for farm to account for the repeated measures on each farm. Initially, a mixed logistic regression model was constructed with glmmTMB (Brooks *et al.* 2017) with a term for the serial autocorrelation of repeated measurements, but this model showed poor fit to the data, as indicated by residual patterns suggesting biased estimates. Generalised estimating equations had the same limitations. We ultimately constructed a Bayesian logistic regression model with dichotomised APC as the response variable and log_{10} SCC as the explanatory variable, and a random intercept for farm to account for the repeated measures on each farm. Using a null (intercept only) model, an AR1 autoregressive correlation structure was compared to the same model without an autocorrelation structure (both had a random intercept for farm). A quadratic term for log_{10} SCC and a random slope for each farm were appraised by comparing the fit of nested models. All models were run with four chains of 2,000 iterations each, with a burn-in of 1,000

iterations and a thinning rate of 1 using the brms package (Bürkner 2021). Weakly informative priors were used. The fixed effects prior had a normal distribution with mean 0 and SD 2, and the random intercept prior had a Student t distribution with three degrees of freedom, a location parameter of 0, and a scale parameter of 2.5. The intraclass correlation coefficient (ICC), which is the proportion of the total variance that is attributable to the farm level, was calculated with the latent variable method (Wu *et al.* 2012).

Models were checked for convergence and mixing by inspecting R-hat 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, which may indicate model misspecification, were checked by computing Pareto *k* values, with values >0.7 indicating potential issues. The statistical significance of the association between $\log_{10}$ SCC and $\log_{10}$ APC was determined by leave-one-out cross-validation (LOO-CV) in comparison to an analogous model without the fixed effect. 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 two times the SE of the difference taken as significant.

Marginal predicted probabilities were taken from the median of the posterior distribution of the model, and 95% credible intervals were calculated from the 2.5^{th} and 97.5^{th} percentiles of the posterior distribution. The predicted probabilities were plotted against $\log_{10}$ SCC, with the raw data superimposed. To determine the risk difference between different values of $\log_{10}$ SCC, contrasts were calculated from the posterior distribution of the model, using SCC values of 500,000, 750,000, and 1,000,000 compared to a reference SCC of 250,000

cells/mL. The distributions of the contrasts were plotted, and the median and 95% CIs were calculated.

7.4 Results

Data from 907 bulk milk consignments were provided, collected between 03 August 2022 and 05 May 2023. The number of bulk milk test results per farm ranged from 6 to 78, with a median of 70. Somatic cell count and APC were missing for 4 consignments from 4 farms and 11 consignments from 7 farms, respectively. All consignments had at least one of SCC or APC results available.

7.4.1 Somatic cell count and aerobic plate count

Overall, SCC had a bimodal distribution, with a median (range) of 649,000 (205,000 to 2,481,000) cells/mL, and a geometric mean of 659,491 (95% CI: 639,068-680,567) cells/mL (Figure 1A). Somatic cell count exceeded 500,000, 1,000,000 and 1,500,000 cells/mL in 640/903 (70.9%), 219/903 (24.3%) and 32/903 (3.5%) of consignments, respectively. There was no clear pattern in the distribution of SCC across time (Figure 2A).

The distributions of $\log_{10}$ APC overall and across time are shown in Figures 1B and 2B, respectively. Aerobic plate count exceeded 100,000 CFU/mL in 200/896 (22.3, 95% CI: 19.7–25.2%) consignments. When plotted by calendar month, APC peaked in August and generally declined across the season (Figure 3).

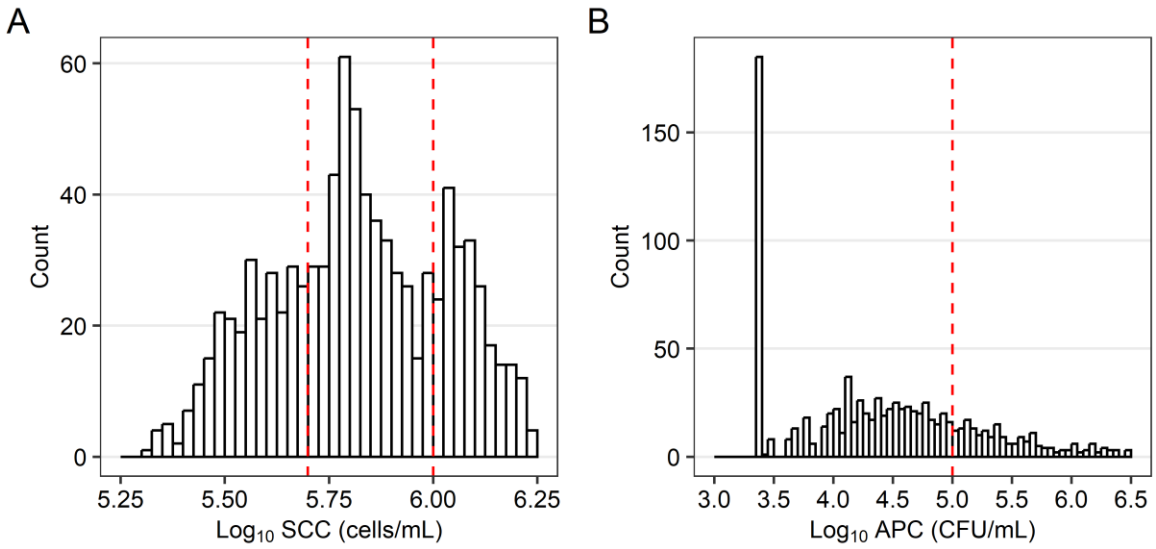

Figure 1: Distributions of A) log_{10} bulk milk somatic cell count (SCC), and B) log_{10} aerobic plate count (APC), in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. Dashed vertical lines represent SCC of 500,000 and 1,000,000 cells/mL and APC of 100,000 CFU/mL.

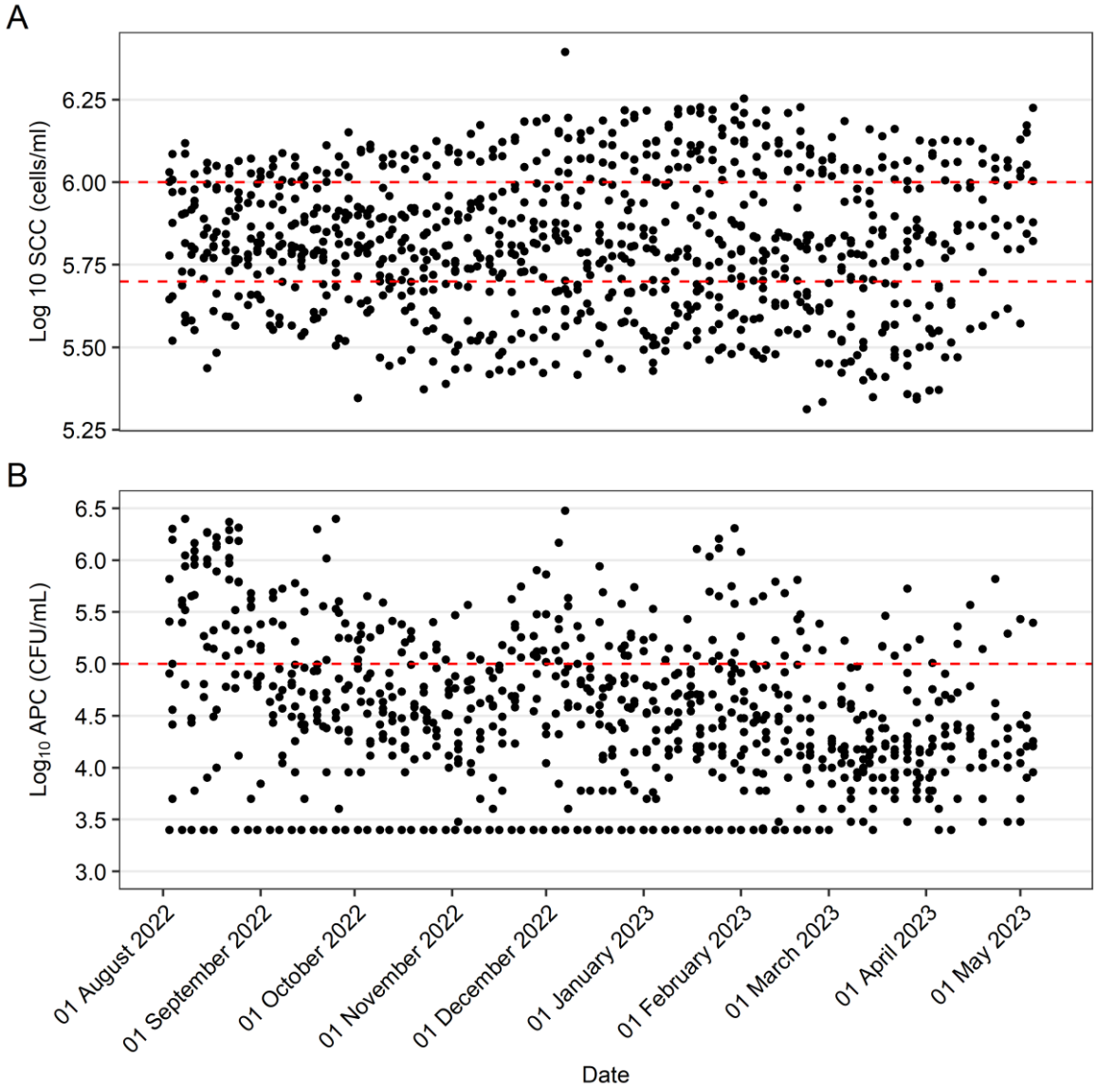

Figure 2: Distributions of A) log_{10} bulk milk somatic cell count (SCC) and B) log_{10} aerobic plate count (APC), across the season in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. Dashed horizontal lines represent SCCs of 500,000 and 1,000,000 cells/mL, and APC of 100,000 CFU/mL.

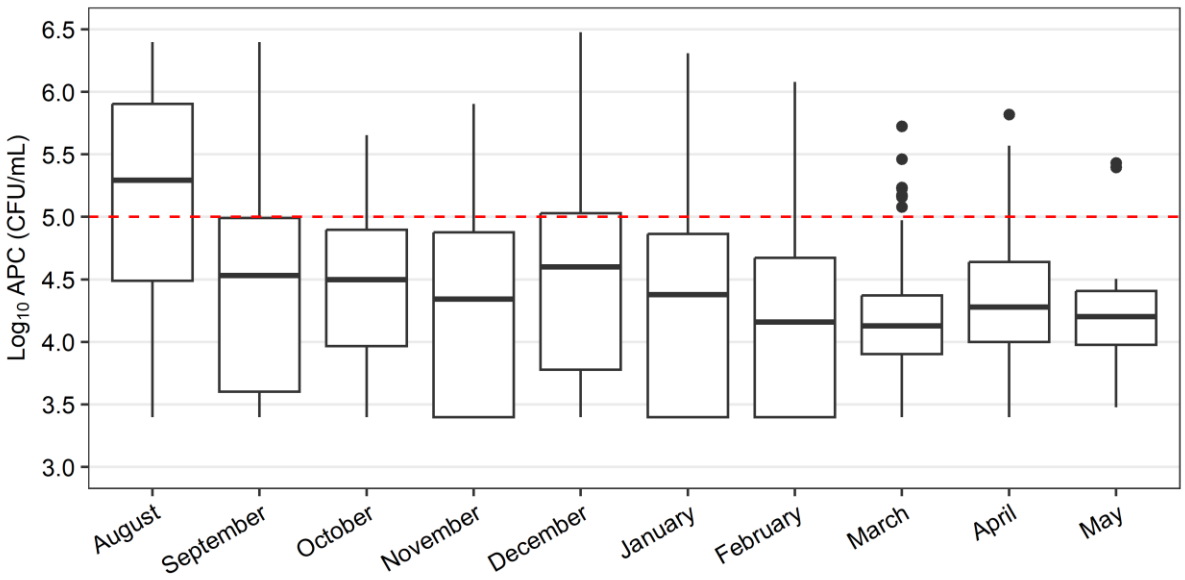

Figure 3: Distribution of log_{10} bulk milk aerobic plate count in each calendar month of the 2022-2023 season, in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. The dashed horizontal line indicates an APC of 100,000 CFU/mL. Boxes extend from the first to the third quartiles, 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 interquartile range from the third quartile. Lower whiskers extend from the first quartile to the lowest value that is no more than 1.5 times the interquartile range from the first quartile. Data beyond the end of the whiskers are deemed outliers and are plotted individually.

At the farm level, geometric mean season BMSCC ranged from 358,198 to 1,278,382, and the proportion of consignments exceeding an APC of 100,000 CFU/mL ranged from 0% to 100% (Table 1). The time series of log_{10} SCC and log_{10} APC on each farm illustrate greater variability in APC than in SCC (Figure 4).

Table 1: Farm-level bulk milk somatic cell count (SCC, cells/mL) and aerobic plate count (APC, CFU/mL) in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. The farms are ordered on geometric mean SCC.

Farm	SCC					APC	
	N	Mean (SD) log_{10} SCC	Geometric mean	N (%) >500,000	N (%) >1,000,000	N	N (%) >100,000
B	70	5.55 (0.12)	358,198	9 (12.9%)	0 (0%)	70	3 (4.3%)
K	78	5.57 (0.08)	375,674	3 (3.8%)	0 (0%)	75	5 (6.4%)
H	67	5.58 (0.13)	376,688	14 (20.9%)	0 (0%)	67	6 (9%)
P	5	5.58 (0.06)	378,208	0 (0%)	0 (0%)	6	6 (100%)
G	70	5.7 (0.07)	502,342	40 (57.1%)	0 (0%)	70	2 (2.9%)
O	8	5.71 (0.14)	509,113	5 (55.6%)	0 (0%)	9	5 (55.6%)
A	69	5.75 (0.13)	556,745	50 (72.5%)	2 (2.9%)	69	15 (21.7%)
D	78	5.81 (0.06)	645,945	76 (97.4%)	2 (2.6%)	77	19 (24.4%)
M	64	5.82 (0.11)	661,861	55 (85.9%)	3 (4.7%)	64	28 (43.8%)
T	11	5.83 (0.06)	671,732	11 (91.7%)	0 (0%)	11	1 (8.3%)
J	71	5.86 (0.08)	719,752	68 (95.8%)	4 (5.6%)	71	19 (26.8%)
L	76	5.94 (0.1)	862,468	75 (98.7%)	23 (30.3%)	75	18 (23.7%)
F	78	6.01 (0.09)	1,021,107	77 (98.7%)	43 (55.1%)	77	19 (24.4%)
R	6	6.01 (0.28)	1,034,332	5 (71.4%)	4 (57.1%)	7	2 (28.6%)
C	76	6.09 (0.07)	1,227,506	76 (100%)	69 (90.8%)	74	22 (28.9%)
N	76	6.11 (0.08)	1,278,382	76 (100%)	69 (90.8%)	74	30 (39.5%)

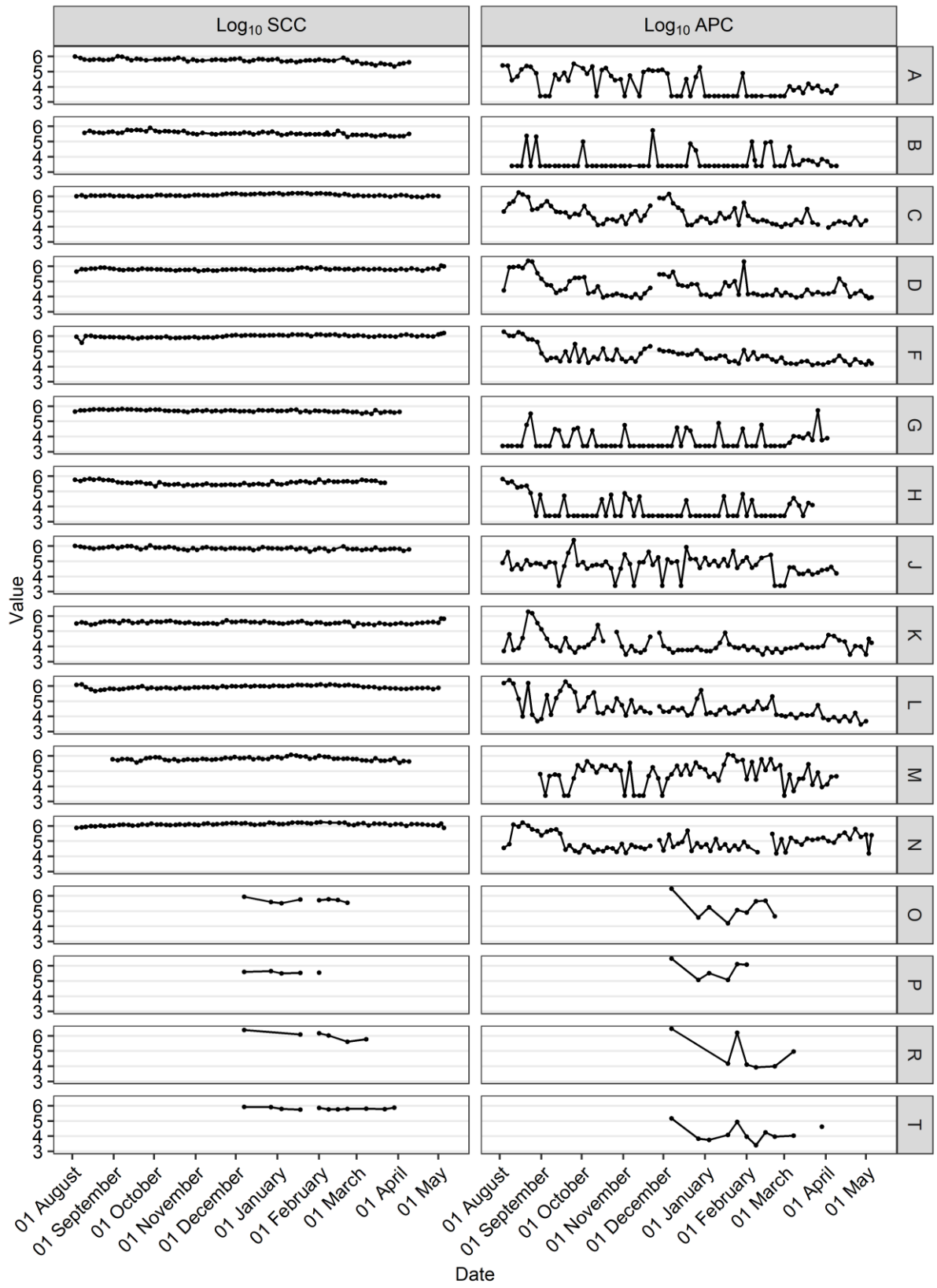

Figure 4: Time series of log_{10} aerobic plate count (APC) and log_{10} bulk milk somatic cell count (SCC) on each farm (A-T) in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand.

The four farms in the lowest quarter for SCC (i.e., the four farms with the lowest season mean log_{10} SCC: farms B, K, H and P) had a mean (SD) log_{10} SCC of 5.6 (0.1) and a geometric mean of 370,383 (95% CI: 358,306-382,868) cells/mL, compared to 6.1 (0.1) and a geometric mean of 1,165,166 (95% CI: 1,131,346-1,199,997) cells/mL for the highest quarter. The proportion of consignments exceeding 100,000 CFU/mL for the four farms in the lowest quarter for APC (i.e., the four farms with the lowest proportion of consignments exceeding 100,000 CFU/mL: farms G, B, K and T) was 11/230 (4.8, 95% CI: 2.7–8.4%), compared to 69/155 (44.5, 95% CI: 36.9–52.4%) for the highest quarter. Two farms were in the lowest quarter for both SCC and APC.

7.4.2 Relationship between somatic cell count and aerobic plate count

Log_{10} SCC and log_{10} APC had a positive relationship (Figure 5). Across all four seasonal periods, linear regression lines indicated a consistently positive relationship (an upward slope from left to right) between SCC and APC (Figure 6). On the other hand, the relationship was not consistent across farms, with 3/16 farms (farms F, N and R) having a negative correlation (the regression line had a downward slope from left to right) between log_{10} SCC and log_{10} APC (Figure 7). Farms O, P, R and T only had 5-10 bulk milk consignments, limiting the ability to draw a clear relationship.

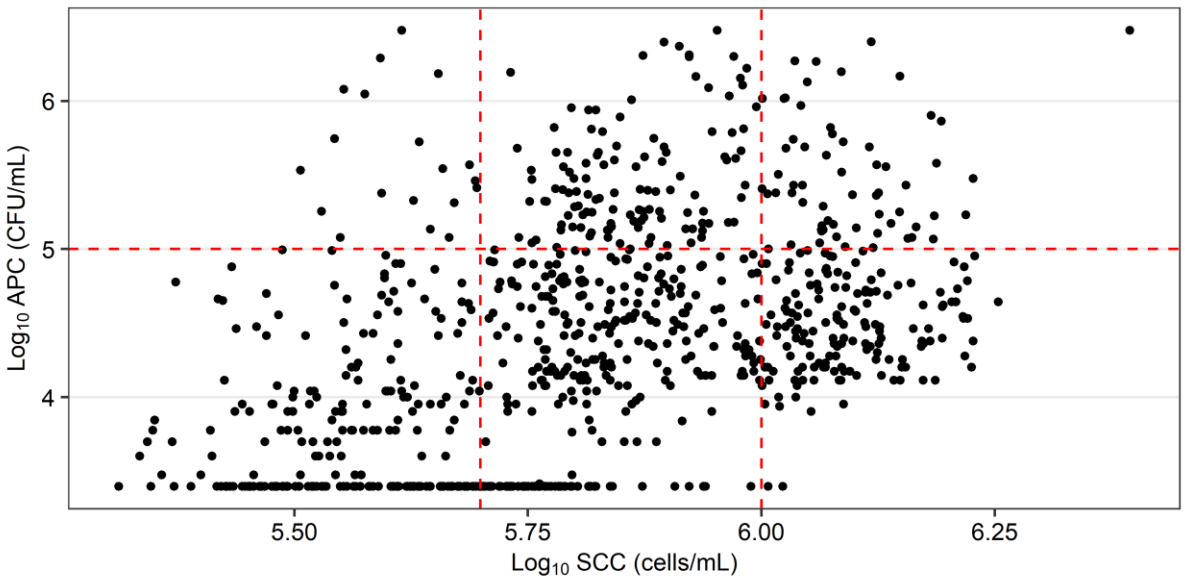

Figure 5: Scatterplot of log_{10} bulk milk somatic cell count (SCC) versus log_{10} aerobic plate count (APC) in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. The dashed horizontal line indicates an APC of 100,000 CFU/mL and the dashed vertical lines indicate SCCs of 500,000 and 1,000,000 cells/mL.

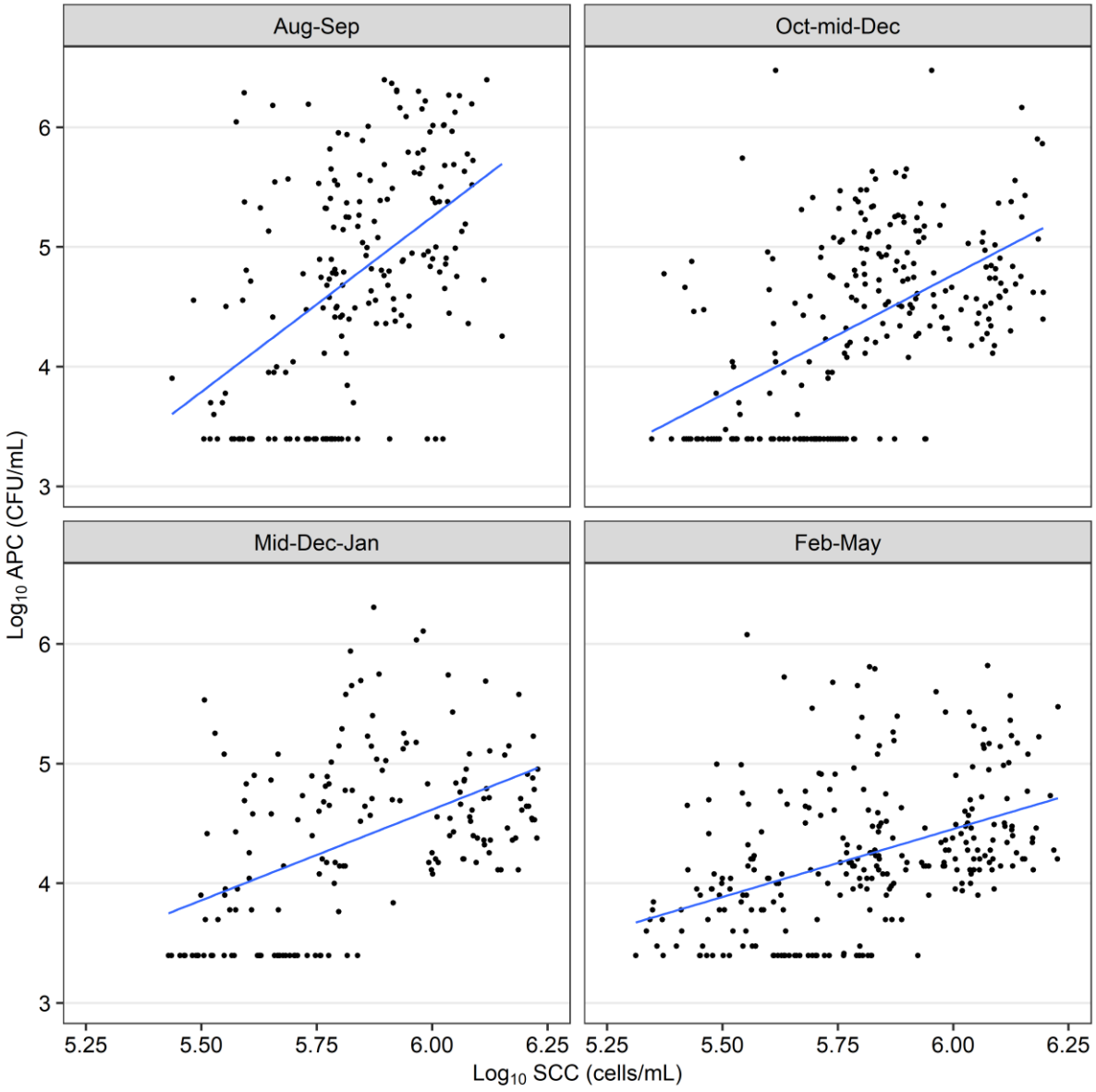

Figure 6: Scatterplot of log_{10} bulk milk somatic cell count (SCC) versus log_{10} aerobic plate count (APC), over four time periods of the 2022-2023 season, in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. The blue lines are linear regression lines.

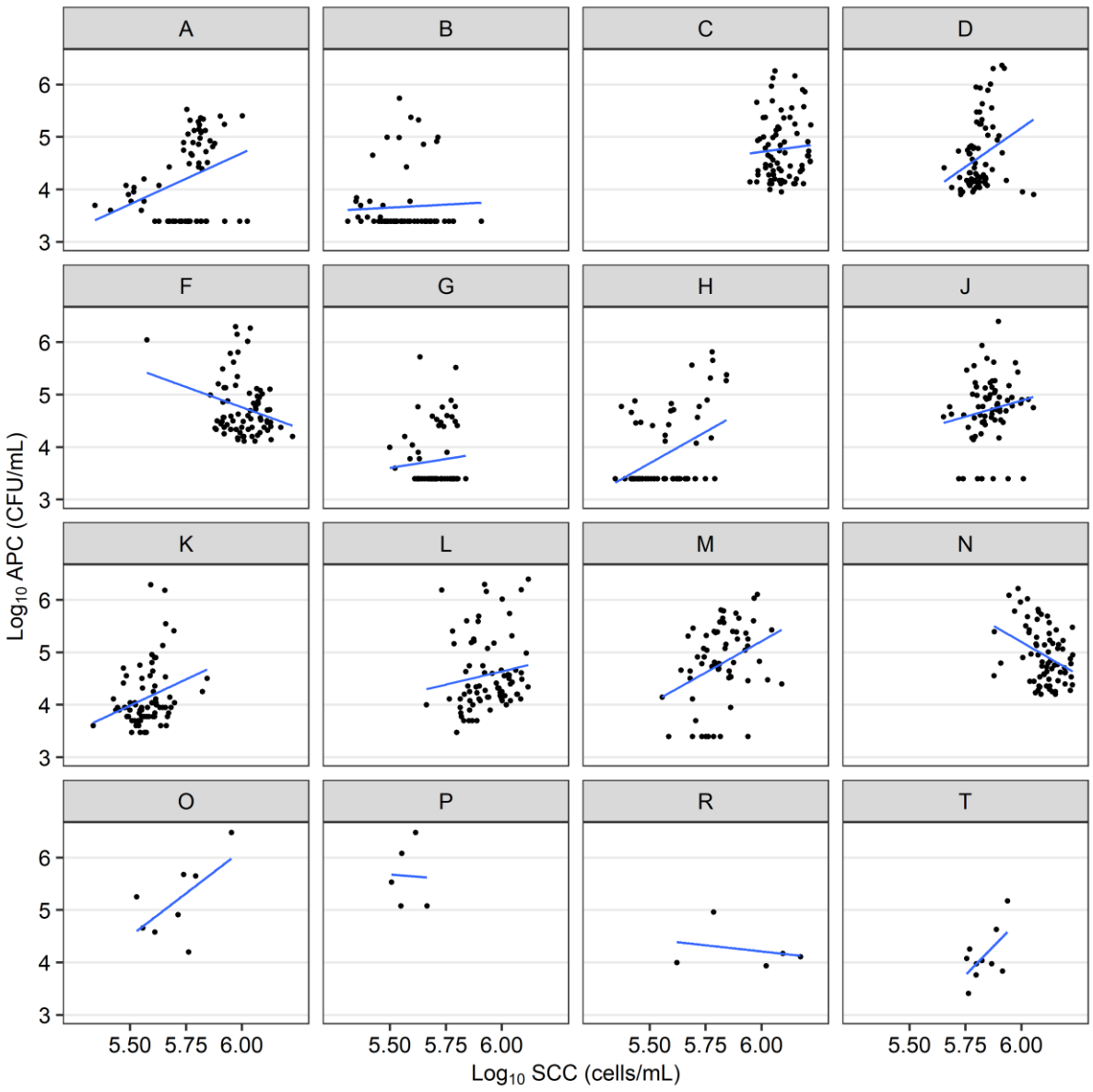

Figure 7: Scatterplot of log_{10} bulk milk somatic cell count (SCC) versus log_{10} aerobic plate count (APC) by farm in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. The blue lines are linear regression lines.

The correlation between log_{10} SCC and log_{10} APC was 0.49 (95% CI: 0.44-0.54) using the Spearman's rank correlation coefficient, but accounting for clustering resulted in a lower repeated measures correlation coefficient of 0.18 (95% CI: 0.11-0.24).

The final regression model contained a fixed effect for log_{10} SCC and a random intercept for farm. Log_{10} SCC had a coefficient of 2.46 (95% CI: 1.07-3.94) on the logit scale. This corresponds to a 1-unit increase in SCC on the log_{10} scale (i.e., a 10-fold increase on the

natural scale) being estimated to increase the odds of APC exceeding 100,000 CFU/mL by a factor of 11.66 (95% CI: 2.92-51.67), and doubling the SCC was associated with approximately double the odds of APC exceeding 100,000 CFU/mL, (OR = 2.09, 95% CI = 1.38-3.28). While the 95% credible interval of the fixed effect for $\log_{10}$ SCC did not include zero, the model did not fit the data significantly better than the null model without $\log_{10}$ SCC (ELPD difference = 3.32, SE = 2.98), and the conditional and marginal model R^2 values were 0.106 (95% CI: 0.071-0.143) and 0.042 (95% CI: 0.001-0.099), respectively. The ICC was 0.32. The model met all diagnostic criteria. Including an autoregressive correlation structure did not improve the model fit, nor did random slopes (for each farm) or a square term for $\log_{10}$ SCC.

Predicted probabilities of APC exceeding 100,000 CFU/mL for different values of $\log_{10}$ SCC are shown in Figure 8, with the raw data and probability curve superimposed. The predicted probabilities were 0.17 (95% CI: 0.1-0.28) and 0.3 (95% CI: 0.18-0.45) for SCC of 500,000 and 1,000,000 cells/mL, respectively. Figure 9 shows the posterior distribution of the contrasts between the predicted probabilities for SCC values of 500,000, 750,000, and 1,000,000 cells/mL and a SCC of 250,000 cells/mL. As the SCC increases, the centre of the risk difference increases, but there is substantial overlap between distributions. The median (95% CI) risk differences were 0.08 (0.04-0.12) for a SCC of 500,000 cells/mL, 0.14 (0.07-0.24) for a SCC of 750,000 cells/mL, and 0.2 (0.09-0.34) for a SCC of 1,000,000 cells/mL.

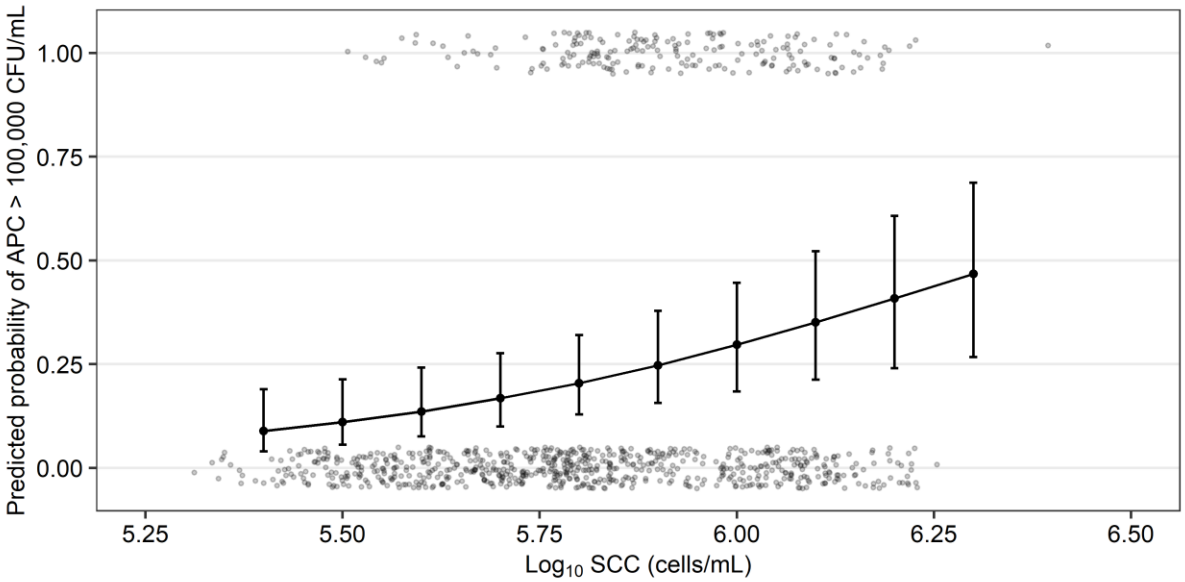

Figure 8: Predicted probabilities of aerobic plate count (APC) exceeding 100,000 CFU/mL for different values of log_{10} bulk milk somatic cell count (SCC), with raw data superimposed, in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. Error bars represent 95% credible intervals.

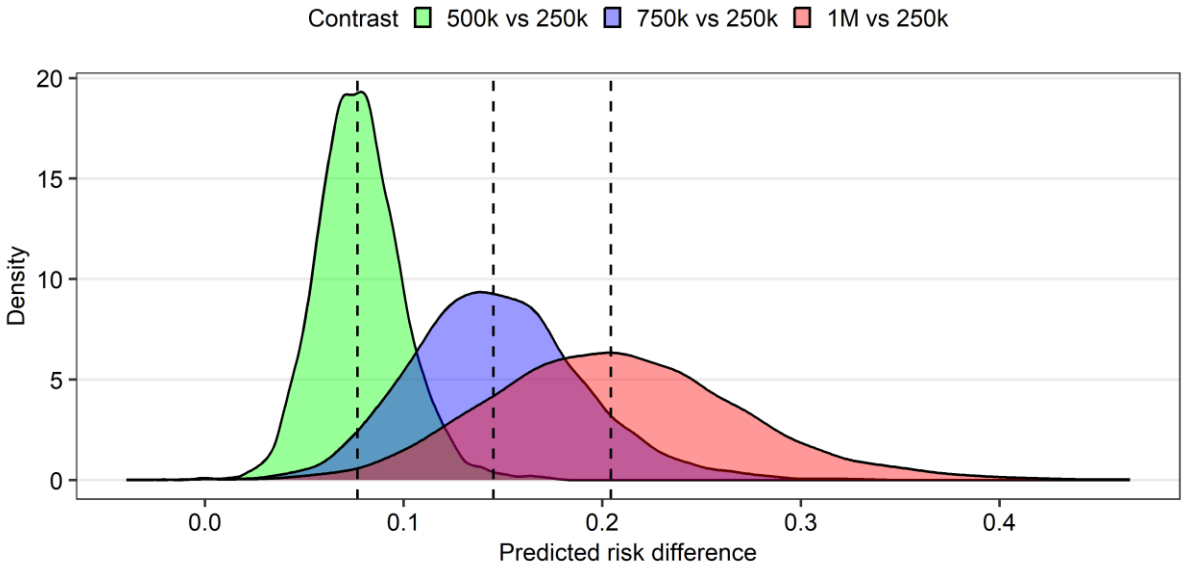

Figure 9: Posterior distributions of the predicted risk difference of bulk milk aerobic plate count (APC) exceeding 100,000 CFU/mL, comparing higher bulk milk somatic cell counts (SCC) to 250,000 cells/mL, in a study of bulk milk quality on 16 commercial dairy sheep farms in New Zealand. Distributions represent draws from the posterior predictive distribution of a Bayesian logistic regression model for each contrast. Dashed vertical lines indicate the posterior median risk difference. For example, the green density corresponds to the predicted risk difference between 500,000 and 250,000 cells/mL. A positive value indicates increased predicted risk at the higher SCC level.

7.5 Discussion

Our data were collected from commercial sheep dairy farms over the 2022-2023 season and reflect the bulk milk SCC and APC of a large sample of New Zealand dairy sheep farms. We used robust statistical methods to explore the relationship between SCC and APC. These data establish a baseline for understanding bulk milk quality in New Zealand dairy sheep farms.

The geometric mean BMSCC of 659,491 cells/mL was well below the MPI threshold for BMSCC of raw sheep milk of 1,500,000 cells/mL (Anonymous 2025), but 24.3% of consignments were >1,000,000 and 3.5% were >1,500,000 cells/mL. This geometric mean was higher than the 488,000 cells/mL reported by Lianou *et al*. (2021) in their study of 325 Greek flocks. Geometric mean BMSCC exceeded 1,000,000 cells/mL on 4/16 (25%) of farms in the present study, compared to 54/325 (16.6%) of farms in the Greek study. In contrast, using monthly BMSCC data from 340 Spanish flocks over 12 months, Gonzalo *et al*. (2019) reported the geometric mean BMSCC to be 1,072,000 cells/mL, while de Garnica *et al*. (2013), using data from 205 Spanish flocks sampled at four time points across one season, reported a geometric mean of 1,093,000 cells/mL.

Bulk milk SCC is a function of the SCC and milk volume of each ewe contributing to the bulk milk. In a separate arm of the same research project as the present study, composite milk samples were collected from approximately 15 randomly selected ewes from all 20 farms at three time points in the season. The geometric mean SCC of these 890 samples was substantially lower at 169,039 (95% CI: 153,921-185,641) cells/mL (Chambers *et al.* 2025c). The difference may be because BMSCC is a bulk measure, which can be disproportionately influenced by a small number of ewes with very high SCC. This effect may be further amplified if those ewes contribute relatively large milk volumes.

There was substantial variation between farms, with geometric mean BMSCC ranging from 358,198 to 1,278,382 cells/mL. We did not investigate factors associated with BMSCC, but the data indicate that relatively low BMSCCs can be achieved under commercial conditions. Differences between farms in the use of routine flock testing may explain some of the between-farm variation, as well as variable application of post-milking teat disinfection and maintenance of optimal milking plant settings, which have been associated with lower BMSCC (Gonzalo *et al.* 2019).

There are no industry-wide targets for BMSCC to our knowledge. Using a similar approach to the bovine dairy industry (Brownlie *et al.* 2014), we suggest that a target of 400,000 cells/mL, based on the geometric mean of 370,383 cells/mL from the lowest quarter of farms in our study, could be used. Routine flock testing may assist farmers in identifying high-SCC ewes and managing BMSCC, while post-milking teat disinfection and maintaining optimal milking plant settings are likely to prevent elevated SCC (Gonzalo *et al.* 2019).

Compared to SCC, APC was volatile and varied across a wider range. It was highest in August, coinciding with the first month of lactation, which may be related to rainfall and wet underfoot conditions during this period. Wet weather can increase APC by increasing mud, faecal contamination and moisture on the udder, teats and surrounding fleece, thereby increasing the bacterial load on teat skin and teat ends before milking. Moisture may also prolong bacterial survival and make pre-milking teat preparation less effective, increasing the likelihood that environmental bacteria enter milk during suckling, milking or sample collection. Lianou *et al*. (2021) found total bacterial count (TBC) of bulk milk to be highest in the first month of lactation, which, in the system they studied, is typically in autumn or winter, when animals spend more time indoors and conditions can be more crowded, resulting in greater teat contamination.

Because APC was censored at laboratory detection limits and the laboratories used different censoring thresholds, APC was not a truly continuous variable. We therefore did not report measures of central tendency such as the mean or median. Instead, for the primary analysis, we reported the proportion of consignments exceeding a threshold of 100,000 CFU/mL, the MPI action limit for APC in raw sheep milk (Anonymous 2025). This threshold-based outcome was clear, industry-relevant and directly interpretable by farmers and processors. However, dichotomising APC resulted in loss of information, particularly when evaluating its relationship with BMSCC, because values just below and far below the action limit were treated alike, as were values just above and far above it. To partly address this, we also explored the SCC–APC relationship graphically and using correlation methods with $\log_{10}$ APC treated as a continuous variable, before using a logistic regression model with dichotomised APC as the technically more robust analysis for the censored data. This approach confirmed that SCC and APC were positively associated, but that BMSCC had limited predictive value for APC exceeding 100,000 CFU/mL. Future studies should ideally measure APC over a non-censored range, allowing the relationship between APC and BMSCC to be analysed without dichotomisation.

The APC threshold of 100,000 CFU/mL was more challenging to meet than the SCC threshold, with 22.3% of consignments exceeding it. It is typical for bulk milk to be collected every three to four days from dairy sheep farms in New Zealand, so the prolonged storage time may exacerbate higher bacterial loads. However, Lianou *et al*. (2021) reported 17.9% of flocks to have a TBC >1,500,000 CFU/mL (based on a single sample from each farm), while Gonzalo *et al*. (2019) reported a geometric mean TBC of 111,000 CFU/mL (based on monthly samples across one season) and de Garnica *et al*. (2013) reported a geometric mean TBC of 136,000 CFU/mL (based on four samples per farm across one season). These studies imply that APC was relatively low in the New Zealand flocks included in the present study.

There was again substantial between-farm variation, with the proportion of consignments exceeding 100,000 CFU/mL ranging from 2.9% to 100%. While this implies on-farm factors influence APC, such as mastitis prevalence (Chambers *et al.* 2025b), and hygiene, bulk tank temperature and plant settings (Gonzalo *et al.* 2019), handling of milk during transport (hygiene and temperature) to the processor may also have influenced APC if the milk sample is taken upon arrival at the processor instead of directly from the bulk milk vat on farm. Chambers *et al*. (2025b) suggest that mastitis may be a contributor to high APC in dairy sheep. However, only 3.1% of the composite milk samples collected by Chambers *et al*. (2025b) had an APC >100,000 CFU/mL, indicating that individual high-APC ewes are unlikely to be the sole explanation for elevated bulk milk APC on most farms. Farmers managing elevated APC should rule out established causes of elevated APC related to milking hygiene, plant sanitation and refrigeration (Jayarao *et al.* 2004), while also considering flock-level screening for high-SCC of high-APC ewes, particularly if routine troubleshooting does not resolve the issue. Further work using repeated concurrent ewe-level, bulk-milk sampling would help clarify the relative importance of mastitis, teat contamination, plant hygiene, refrigeration and milk transport.

None of the farms in our study avoided an APC exceeding 100,000 CFU/mL across the whole milking season, but only 4.8% of consignments exceeded this threshold on the four farms in the lowest quarter for APC. We suggest a target of <5% of consignments exceeding 100,000 CFU/mL.

We present four assessments of the relationship between SCC and APC and show that the method is important because the relationship became weaker as we moved from less robust to more robust methods. The first assessment was a simple scatter plot, which showed a positive relationship between $\log_{10}$ SCC and $\log_{10}$ APC. The second assessment was Spearman's rank correlation coefficient, which confirmed the positive relationship, but did not account for

multiple measurements per farm. The third assessment was the repeated measures correlation coefficient, which accounted for the multiple measurements, and doing so reduced the correlation coefficient from 0.49 (95% CI: 0.44-0.54) to 0.18 (95% CI: 0.11-0.24). Repeated-measures correlation assumes a common slope across farms, and that may not hold if the drivers of APC differ between farms. A mixed-effects approach with random slopes would be one way to relax that assumption, but we did not take that approach because the primary aim of the chapter was to estimate an overall industry-level relationship rather than farm-specific relationships. The fourth assessment was a regression model that included a fixed effect for $\log_{10}$ SCC and a random intercept for farm. This confirmed an association but showed that SCC has poor predictive power for APC exceeding 100,000 CFU/mL.

The first three methods treated APC as a continuous variable but should only be regarded as exploratory methods because APC is a censored variable. We therefore dichotomised APC and used a logistic regression model. This approach also accounted for clustering by farm, since observations within a farm are not independent and therefore the study power is less than a naive approach would assume. With an ICC of 0.32, one third of the variance in dichotomised APC was explained by farm-level factors, and two thirds by within-farm factors, meaning that high APC was strongly clustered within farms. This four-step process of appraising the relationship between SCC and APC shows the importance of applying robust statistical techniques to data that are not continuous and are not independent.

While the final model confirmed a positive association between $\log_{10}$ SCC and the log-odds of APC >100,000 CFU/mL, it did not fit the data significantly better than the null model without $\log_{10}$ SCC. Furthermore, the conditional and marginal R^2 values were low. Together, these results indicate that APC >100,000 CFU/mL is more strongly associated with unmeasured farm-level factors than with BMSCC, as can be seen in the varying slopes in Figure 7. Although a relationship exists between SCC and APC, and, on average, the

probability of APC >100,000 CFU/mL increases with higher SCC, the relationship is relatively weak in the context of the large variation in APC. This is supported by Figure 4, which shows the relatively large variance in APC compared to SCC on each farm, Figure 7, which shows that SCC and APC did not co-vary in a consistent manner within farm (and three farms had a negative relationship between $\log_{10}$ SCC and $\log_{10}$ APC), and Figure 9, which shows the large overlap in predicted risk of APC >100,000 CFU/mL for SCCs of 500,000, 750,000 and 1,000,000 cells/mL. Mastitis may be a contributor to high APC, and managing SCC (and therefore mastitis) may control APC, but there are clearly unrecorded on-farm factors that more strongly predict high APC on at least some farms, such as milking hygiene, plant sanitation, and refrigeration, as well as transport to the processor. Further work is needed to clarify if bulk milk APC is driven by mastitis on farms with a strong positive relationship between bulk milk APC and SCC, but by other factors (such as refrigeration and hygiene) when there is no or a negative relationship. Some predictive power may have been lost in the dichotomisation of APC, since the APC values move from a spectrum to either above or below the threshold, but a non-censored measure of APC is needed to confirm that.

Other researchers have inferred a positive relationship between SCC and APC in dairy sheep. Pearson correlation coefficients of 0.269 across 325 Greek farms (Lianou *et al.* 2021) and 0.42 across 205 Spanish farms (de Garnica *et al.* 2013) were reported between SCC and TBC in bulk milk, though the consistency of the relationship across farms was not reported in either study. At the individual ewe level, Chambers *et al*. (2025b) found that elevated rapid mastitis test score (score 2-3 on a scale of 0, trace, 1, 2, and 3) and $\log_{10}$ SCC were risk factors for elevated APC.

Our study only reported bulk milk SCC and APC data that were already collected routinely by the processors and is limited by small sample sizes for some farms. On 12/16 farms, 67-78 samples were collected, while on 4/12 farms only 5-11 samples were collected. The study

was retrospective and cross sectional in nature, and therefore can only be used to infer association, not causation. There is no national register of bulk milk test data, so we relied on a purposive sample of farms, which may not represent the wider industry. However, we sampled approximately 50% of the commercial farms estimated to have been operating at the time.

We recommend further work to investigate factors that influence bulk milk SCC and APC, such as weather, teat hygiene and milking plant specifications, whether management interventions can change SCC and APC, and follow up descriptive work to see if these parameters change over time as the relatively new industry matures. Ideally, future research would measure APC on a truly continuous scale so that the relationship between SCC and APC can be explored without the limitations of a dichotomised variable.

We proposed targets for bulk milk SCC and APC based on this work. Our intention is to set an aspirational goal for farmers willing to benchmark against the best-performing farms and drive continuous improvement in milk quality. These targets, <400,000 cells/mL for geometric mean BMSCC and <5% of consignments exceeding 100,000 CFU/mL for APC, represent what is already achieved by the top quarter of farms in this study, and therefore are ambitious but demonstrably attainable. Framing these benchmarks as aspirational acknowledges that they may not be immediately achievable for all producers but provides a practical reference point for farmers, advisors, and industry stakeholders aiming to improve milk quality through ongoing management refinement and investment in infrastructure, hygiene, and animal health. While further research is required to elucidate the factors contributing to bulk milk SCC and APC on New Zealand farms, these targets are likely to be achieved by implementing routine milk recording to detect ewes with high SCC (and therefore potentially high APC) and careful attention to mastitis detection (Chambers *et al.*

2025b) and milking hygiene practices and plant maintenance (Gonzalo *et al.* 2019) to manage APC.

7.6 Conclusions

Bulk milk SCC had a geometric mean of 659,491 (95% CI: 639,068-680,567) cells/mL and varied widely between farms. Aerobic plate count exceeded 100,000 CFU/mL in 200/896 (22.3, 95% CI: 19.7–25.2%) consignments, appeared to be highest in August, and the proportion exceeding this threshold varied widely between farms. There was a positive relationship between SCC and APC, but the relationship was weak and not consistent across farms. Ranking the 16 farms on SCC, the geometric mean SCC of the best quarter of farms was 370,383 (95% CI: 358,306-382,868) cells/mL. Ranking the farms on the proportion of consignments exceeding 100,000 CFU/mL, the best quarter of farms had 11/230 (4.8, 95% CI: 2.7–8.4%) consignments exceeding this threshold. We propose bulk milk SCC and APC targets of a geometric mean of 400,000 cells/mL and <5% of consignments exceeding 100,000 CFU/mL, respectively. The results provide a baseline for bulk milk quality on New Zealand dairy sheep farms and suggest that while SCC is positively associated with APC, it is not a reliable predictor of APC. Further research should investigate the factors influencing APC and to develop strategies for managing it effectively.

7.7 Acknowledgements

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8 MASTITIS CONTROL IN NEW ZEALAND DAIRY SHEEP: RECOMMENDED BENCHMARKS, PRACTICES AND RESEARCH PRIORITIES FROM A BASELINE NATIONAL RESEARCH PROGRAMME

The last of the chapters to be submitted to a scientific journal, Chapter 8 differs from the previous chapters by summarising the research and making recommendations for the future. It is a narrative review that lays out benchmarks based on the best-performing farms, recommends the practices needed to advance the New Zealand dairy sheep industry in terms of mastitis and milk quality, and briefly summarises the research that is needed to address the remaining gaps in our knowledge. It prioritises these into immediate, short-term, and medium-term recommendations.

Chapter 8 was submitted to the New Zealand Veterinary Journal as a Review article on 4 December 2025.

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Mastitis control in New Zealand dairy sheep: recommended benchmarks, practices and research priorities from a baseline national research programme

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8.1 Abstract

Sheep milking in New Zealand has grown rapidly in the last 20 years. In the 2022-2023 season, research project conducted on 20 commercial farms (approximately 50% of the industry) established baseline parameters for udder health and milk quality. This paper synthesises findings from that project alongside relevant international dairy sheep mastitis literature to clarify what is known, what is uncertain, and what actions are most likely to accelerate progress in New Zealand. It proposes immediate, short-term, and medium-term priorities for mastitis control in New Zealand dairy sheep. We set benchmarks for clinical mastitis incidence (<1.5%), subclinical mastitis prevalence (<2%), and bulk-milk aerobic plate count (<5% consignments ≥100,000 CFU/mL) and somatic cell count (season mean <400,000 cells/mL) using the mean of the best 25% (best quarter) of farms. On-farm practices most likely to achieve these benchmarks are summarised, including milk recording, use of the rapid mastitis test, somatic cell count thresholds, milking hygiene methods and udder conformation scoring. Research priorities include determining the factors associated with high bulk-milk aerobic plate count, clarifying mastitis identification and management when multiple milk-recording events take place within a season, extending the preliminary

investigation of mastitis risk factors and treatments, confirming the value of milking hygiene practices, and developing a rapid udder-conformation scoring system.

8.2 Introduction

Sheep milking in New Zealand has grown in the last 20 years from a small number of vertically integrated businesses to approximately 30 commercial farms milking approximately 30,000 ewes in 2022 (McCoard *et al.* 2023). Mastitis is globally recognised an important cause of reduced animal health, productivity and milk quality (Jaeggi *et al.* 2003; Leitner *et al.* 2004; Alba *et al.* 2019). As a relatively new industry, little systematic research has been conducted on mastitis in New Zealand. Therefore, in the 2022-2023 season, a 20-farm research project determined the importance of mastitis in New Zealand dairy sheep and established baseline parameters for udder health and milk quality.

This paper draws together the published findings from that research (Chambers *et al*. 2024; Chambers *et al*. 2025; Chambers and Laven 2026; Chambers *et al*. 2026a-c) and critically appraises where the industry stands currently, where the gaps are, and what the opportunities for improvement are. It provides next steps informed by the baseline evidence for the New Zealand industry by proposing aspirational benchmarks, practices and research. Each benchmark is set at the farm level by taking the mean of the best 25% (best quarter) of the 20 farms in the research project, which is ambitious but already achieved by top performers. This approach has precedent in New Zealand agricultural benchmarking. For example, Brownlie *et al*. (2014) note that New Zealand dairy industry reproductive targets were based on the mean performance of the top quartile of herds. This approach was also used in our bulk-milk quality paper (Chambers *et al*. 2026a), ensuring consistency across outputs from the research programme. Clinical mastitis incidence is defined as the number of new cases divided by the number of ewes milked across a season (defined at the flock-level as the

period from the start of lambing to the end of drying-off), and subclinical mastitis prevalence as the number of cases at any time point divided by the number of ewes milked at that time.

8.3 Current understanding of mastitis in New Zealand dairy sheep

While there is a large international body of published dairy sheep mastitis research, its relevance to New Zealand is limited by differences in breeds, environments and farming systems. Literature specifically pertaining to grazing ewes exists from Mediterranean regions such as Sardinia (Cuccuru *et al*. 2011), Italy (Bianchi *et al*. 2016), Spain (Gonzalo *et al*. 2002), and Greece (Vasileiou *et al*. 2019a), but the extent of grazing, when stated, is often seasonal or only allowed during certain times of the day. Overseas, multiple specialist dairy breeds have been developed over decades to centuries. In contrast, to optimise for local conditions, the New Zealand flock has been assembled from dairy (e.g., East Friesian) and non-dairy breeds (e.g., Poll Dorset and Coopworth), often combined with varying proportions and types of overseas dairy genetics (e.g., Lacaune). New Zealand's industry is young, almost entirely grazing-based, and typically uses modern infrastructure (McCoard *et al*. 2023), and machine milking is standard. These differences in farm system, management, and industry stage mean that findings from overseas are not always transferable to New Zealand conditions.

We conducted the first systematic research program on mastitis in New Zealand dairy sheep in the 2022-2023 season. The objectives were to determine the incidence of clinical mastitis, the prevalence of subclinical mastitis and the bacterial causes of both; establish how clinical mastitis is treated and the outcomes that are achieved; characterise udder and teat conformation and health; describe ewe and bulk milk somatic cell counts (SCC) and aerobic plate count (APC); find SCC thresholds for diagnosing intramammary infection; explore the use of the rapid mastitis test (RMT) and its correlation with SCC; identify key risk factors for

mastitis and elevated APC; and determine associations between bulk milk SCC and APC. The findings summarised below are drawn from a series of published studies from this research programme (Chambers et al. 2024; Chambers et al. 2025; Chambers and Laven 2026; Chambers et al. 2026a–c), which are synthesised and interpreted here.

The research was conducted on 20 commercial farms spread from North Waikato to Canterbury, purposively selected to represent a range of farm systems. These 20 farms comprised approximately half of the commercial dairy sheep farms in operation at the time. Farm demographic, geographic and management information was collected by in-person interview with farm owners and managers and reported in Chambers and Laven (2026). Farmer-diagnosed clinical mastitis data (date of diagnosis, ewe demographic information, clinical features, treatments and outcomes) and milk samples were collected by farm staff as cases were diagnosed according to a standardised definition (a change in the appearance of milk and/or signs of inflammation in the gland) (Chambers *et al.* 2024). Bulk-milk SCC and APC data were collected from the 16 farms that sent milk to a processor (Chambers *et al*. 2026a). At three points in the season, coinciding with early, mid and late lactation, approximately 15 ewes were randomly selected on each farm. Teat and udder conformation and pathology observations were recorded (Chambers *et al*. 2025a), and milk samples were collected by trained technicians following a standardised study protocol for RMT, SCC, APC and bacterial culture (Chambers *et al*. 2026b, c). Ewe-level SCC was measured on fresh foremilk samples using a Combifoss 7 (Foss, Cambridge, New Zealand), and APC was measured by MilkTestNZ (Hamilton, New Zealand) using standard laboratory methods. SCC data were log-transformed for analysis where appropriate, consistent with standard practice. Because samples were collected from foremilk rather than the full milk fraction, the SCC results may differ from those obtained from composite milk-recording samples. However, previous work suggests minimal difference between cisternal and alveolar milk SCC in dairy

ewes sampled within 12 hours of milking (McKusick *et al*. 2002). Subclinical mastitis was defined at the ewe level as a ewe having one or two bacteriologically positive glands and a SCC >500,000 cells/mL and/or a RMT score ≥1. Clinical mastitis data were collected for 236 cases and randomly selected ewe data were collected from a total of 893 ewes.

The farm information collected by interview showed that New Zealand dairy sheep farms were relatively young (median four seasons in operation) and highly variable in lamb-removal timing, milk-harvesting period, staffing, parlour configuration, automation, and data recording (Chambers and Laven 2026). Not only was there between-farm variation in practices, but practices often varied within farms across the season according to staff and resource availability. Milk recording was performed on only 7/20 farms, ewe-level data were often limited, and automation was partial, with automatic cup removers present on one-third of farms and no farms using automatic teat sprayers. This diversity is consistent with an emerging pasture-based industry and contrasts with the more established and often more standardised semi-intensive and intensive dairy sheep systems described overseas.

We found that clinical mastitis had a low incidence (2.3%) (Chambers *et al.* 2024), which is consistent with overseas estimates among dairy ewes of 0.9% in Portugal (Queiroga 2017), and 1.7 cases per 100 ewe-months in Jordan (Lafi 2006). However, direct comparison between studies is limited by differences in study design and case definitions. For example, some studies report point prevalence rather than incidence (Quinlivan 1968; Queiroga 2017), while others rely on periodic farm visits (Lafi *et al*. 1998), which may underestimate incidence. Earlier New Zealand work in non-dairy sheep reported a similar point prevalence of 1.7% (Quinlivan 1968) but shares these limitations. Overall, these findings suggest that clinical mastitis incidence in dairy sheep is generally low across systems, although variation between studies may reflect methodological differences as well as true differences in management and production systems.

While the incidence was low, clinical mastitis could be severe, with an overlapping 25% of affected ewes having a fever and 26% having depression, and only 15% of ewes recovering without lasting sequelae. Nearly half of all the clinical mastitis milk samples were culture negative. Including culture-negative samples in the denominator, *Streptococcus uberis* (14%), non-aureus staphylococci (12%), and *Staphylococcus aureus* (11%) were the most common isolates.

The distribution of mastitis pathogens in this study was broadly consistent with reports of clinical mastitis in dairy sheep, where staphylococci are typically the predominant isolates (Bergonier and Berthelot 2003; Mork *et al*. 2007; Queiroga 2017). However, *Streptococcus uberis* was isolated more frequently than in most overseas studies, where it is reported infrequently or not at all. This may reflect differences in production systems, as New Zealand dairy sheep are predominantly managed in outdoor, pasture-based systems. A similar pattern is observed in New Zealand dairy cattle, where *S. uberis* is a common cause of clinical mastitis in grazing herds (Petrovski *et al*. 2009). Direct comparison with some published studies is limited by differences in study design, as many reports collect data from subclinical mastitis cases, bulk milk surveys, or ewes with udder defects rather than clinical mastitis cases. These differences in case definition and sampling strategy are likely to contribute to the variability in reported pathogen distributions. All milk samples were frozen on farm and shipped periodically to the laboratory for culture. While standardised sampling instructions were provided to farmers, variation in sampling and handling between farms, as well as freezing prior to culture, may have affected bacterial recovery.

Turning to the randomly selected milking ewes, teat and udder morphological observations were broadly consistent with overseas dairy sheep studies of teat placement, udder depth, suspension, gland separation, symmetry, and supernumerary teats (de la Fuente *et al*. 1996; Casu *et al*. 2006; Makovický *et al*. 2024), although between-farm variation was evident

(Chambers *et al*. 2025). Pathology, assessed visually and by palpation, was rare, with the prevalence of all conditions <6%.

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Subclinical mastitis had a prevalence of 6.4% (Chambers *et al.* 2026b), which is substantially lower than the 26% prevalence estimated by Greek researchers (Vasileiou *et al.* 2018) and the 34% prevalence estimated by las Heras *et al*. (1999) among Spanish ewes. Milk samples from randomly selected ewes had a median SCC of 128,000, a geometric mean of 169,039, and a range of 2,000 to 34,953,000 cells/mL, while 21.2% of these ewes had a RMT score ≥1 in 1 or both glands (Chambers *et al.* 2026c). The geometric mean SCC was lower than the geometric mean of 539,000 cells/mL in a previous study of New Zealand dairy sheep (McDougall *et al.* 2001), but the samples in the 2001 study were all collected at approximately 40 days postpartum when SCC may be higher. On the other hand, the geometric mean SCC 144,544 (log10 5.16) cells/mL among culture-negative ewes in the present study was substantially higher than the geometric means of 72,444 (log10 4.86) cells/mL (Ariznabarreta *et al.* 2002) and 95,499 (log10 4.98) cells/mL (Gonzalo *et al.* 2002) among culture-negative Spanish ewes. The reasons are unclear but may reflect different management systems (including monthly milk recording in one of the Spanish studies) or a possible effect of freezing on sample viability (freezing of samples in the New Zealand study

may have caused some false negative bacteriological diagnoses and therefore inflated the SCC of culture-negative samples). Bulk milk SCC had a geometric mean of 659,491 cells/mL (Chambers *et al.* 2026a); higher than the geometric mean of 488,000 cells/mL reported by Lianou *et al*. (2021) in Greece but lower than the 1,072,000 cells/mL reported by Gonzalo *et al*. (2019) and 1,093,000 cells/mL reported by de Garnica *et al*. (2013) in Spain. Bacteria were isolated from 5.5% of the glands of randomly selected ewes (Chambers *et al.* 2026b), with the most common species being non-aureus staphylococci (4.0% of glands) and *S. aureus* (0.6% of glands), consistent with the aetiology in the northern hemisphere (Fthenakis 1994; Vasileiou *et al.* 2018; Michael *et al.* 2023). We confirmed moderate or severe teat end hyperkeratosis and udder asymmetry as risk factors for subclinical mastitis (Chambers *et al.* 2026b). Teat end hyperkeratosis is a known mastitis risk factor in sheep (Vouraki *et al.* 2018), but this was the first report of an association between udder asymmetry and mastitis in dairy ewes to our knowledge.

The diagnostic values of SCC and RMT were established (Chambers *et al.* 2026c). A SCC threshold of approximately 400,000 cells/mL had the greatest accuracy for diagnosing intramammary infection (IMI) using a single SCC measurement, but while it had a specificity of 0.88 (95% CI = 0.85–0.90), its sensitivity was only 0.64 (95% CI = 0.55–0.73) and its positive predictive value was only 0.37 (95% CI = 0.29–0.45) due to the low prevalence of infection. Mean $\log_{10}$ SCC increased linearly with RMT score but its agreement was only moderate (Kendall's tau = 0.47). The SCC threshold was based on a single SCC measurement, but using multiple SCC measurements to determine mastitis status has been recommended (Berthelot *et al.* 2006). Therefore, local research is required to determine whether the same threshold should be applied when there is more than one SCC measurement within a lactation, and what the minimum number of SCC measurements should be.

High and volatile bulk milk APC is a challenge on New Zealand dairy sheep farms, exceeding 100,000 CFU/mL in 22.3% of consignments and peaking in August (Chambers *et al.* 2026a). Our research confirmed that elevated RMT score and SCC, positive milk culture and subclinical mastitis are risk factors for elevated ewe-level APC (Chambers *et al.* 2026c). For bulk milk, high APC was not well predicted by SCC (Chambers *et al.* 2026a).

We collected a large amount of data and set a baseline understanding of mastitis and milk quality in New Zealand dairy ewes. Together, these findings suggest NZ dairy sheep mastitis is currently a low-incidence/prevalence but high-impact condition, with management and surveillance constrained by limited records and screening. The low prevalence and modest isolate numbers mean that causal inference and ranking of intervention priorities remain premature, reinforcing the need for a second phase of surveillance and hypothesis-generating work. Based on what we have learned, future research can be prioritised to address gaps, particularly in relation to causality, risk factors and diagnosis, which our largely descriptive work was not designed to fully support. The immediate priorities below are therefore framed to improve data quality and decision making before more complex objectives are pursued in the short and medium term.

8.4 Immediate priorities (0-12 months)

8.4.1 Benchmarks

We propose targets of <1.5% for clinical mastitis incidence (Chambers *et al.* 2024) and <2% for subclinical mastitis prevalence (Chambers *et al.* 2026b), and bulk milk quality benchmarks of <5% of bulk milk consignments exceeding 100,000 CFU/mL for APC and season mean SCC <400,000 cells/mL (Chambers *et al.* 2026a). Published dairy sheep literature provides relatively little guidance on flock-level target values for these parameters, tending instead to focus on diagnostic or interpretive thresholds. Although based on a modest

national sample, using top-quartile performance provides a pragmatic, achievable target that avoids importing thresholds from different systems overseas.

8.4.2 Practices

Five actions or practices are strongly supported by the baseline findings to achieve these targets. (1) Form an industry working group to coordinate standard operating procedures (SOP), training assets, benchmarking and farmer incentives. Such groups are well-established for the New Zealand bovine dairy industry (DairyNZ and the National Mastitis Advisory Committee). A precedent was set in France with the formation of MAMOVICAP, which was tasked with developing tools for intervention and decision-making for mastitis in sheep and goats (de Cremoux *et al*. 2018). (2) At least one routine milk recording event with SCC measurement per season on dairy sheep farms is suggested to identify ewes with subclinical mastitis. The International Committee for Animal Recording (ICAR) recommends approximately monthly flock recording visits, with official organisations deciding the number of visits per milk flock and milk period (ICAR Wiki 2025). Milk recording was only performed on 7/20 (35%) of study farms (Chambers and Laven 2026), so support and incentives will be needed. (3) Use the RMT to screen ewes at key points for mastitis, such as the transition from the colostrum flock to the milking flock. Although sheep-specific evidence is limited, a similar approach is recommended in New Zealand dairy cattle, where SmartSAMM advises screening with the RMT before cows move from the colostrum herd to the milk supply herd, with positive cows retained for an additional two to four milkings to reduce the risk of elevated bulk-milk SCC (DairyNZ 2013). The evidence also supports the use of the RMT to confirm suspected mastitis cases based on visual signs, acknowledging possible over-diagnosis in farmer-reported clinical mastitis (Chambers *et al.* 2024). New Zealand research confirmed that the RMT correlates well with SCC (Chambers *et al.* 2026c). An industry SOP and short training videos can be developed to support consistent use. (4)

Strengthen ewe-level identification and data capture to enable routine milk recording and management decision making, as ewe-level data were frequently limited on our study farms (Chambers and Laven 2026). Industry support and practical tools should help accelerate implementation. (5) Implement an industry protocol for identifying and managing ewes with suspected clinical or confirmed subclinical mastitis, such as a farmer-driven sequence of using SCC and/or RMT to detect and confirm elevated SCC, followed by targeted milk culture and monitoring of high-SCC ewes (Chambers *et al*. 2026c). This approach is consistent with small-ruminant mastitis-control approaches that use SCC as a decision tool alongside clinical examination rather than making blanket decisions, such as MAMOVICAP (de Cremoux *et al.* 2018). A single-test SCC threshold of 400,000 cells/mL was confirmed as an initial screening threshold (Chambers *et al.* 2026c), however, follow-up testing (e.g., repeat SCC and/or targeted culture), supported by RMT is necessary due to the low positive predictive value of SCC in the context of low subclinical mastitis prevalence. This practice depends on the previous four.

In addition, our research suggests that veterinarians should engage farmers to increase awareness of the severity of clinical mastitis and provide supportive treatment, such as anti-inflammatory drugs. Approximately 25% of ewes with clinical mastitis had fever and a similar (overlapping) proportion were depressed (Chambers *et al.* 2024). The value of non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of bovine clinical mastitis is well-recognised (Li *et al*. 2023).

8.4.3 Research

Chambers *et al*. (2025c) reported that high and volatile bulk milk APC is a challenge on New Zealand dairy sheep farms and that ewes with mastitis have a higher risk of elevated ewe-level APC, but that this was only one of several potential risk factors for elevated bulk-milk

APC. Three research activities are therefore suggested to generate hypotheses about the causes. (1) Sequence microbes isolated from bulk milk samples with high APC and swabs from ewe teats and udders, milking plant and other potential origins to pinpoint the sources of high-APC milk. Similar approaches have been used in dairy cattle, where mastitis-associated streptococci were shown to contribute to elevated bulk-milk APC (Zadoks *et al.* 2004). (2) Log the temperature of bulk milk on several farms to determine if temperature excursions explain high APCs as a first-line audit. Bulk milk is stored on many farms for three to four days before being transported to the processor. With the low storage volumes of sheep milk, maintaining low and stable temperatures without freezing the milk can be a challenge. (3) Conduct a prospective cohort study to investigate associations between management practices, milk storage conditions, udder-health indicators, and bulk-milk APC, similar to the approach used in dairy cows by Cicconi-Hogan *et al*. (2013).

8.5 Short term priorities (12-24 months)

8.5.1 Benchmarks

Develop new benchmarks or targets for factors associated with high bulk milk APC (such as teat and udder hygiene scores) based on the immediate-priority research.

8.5.2 Practices

Publish a bulk milk APC troubleshooting SOP based on the results of research into high bulk milk APC, focusing on the most likely causes first (e.g., check milking procedures and plant hygiene and temperature), and, if unresolved, focusing on less likely causes (e.g., screen the flock for high-SCC/RMT ewes and culture those ewes). Inspecting farm-specific scatter plots of bulk-milk APC and SCC may clarify if there is a clear positive relationship between the two measures and thus whether to focus on mastitis first or not (Chambers *et al.* 2026a). Trace element testing plans are indicated because selenium deficiency has been associated

with higher clinical mastitis risk (Giadinis *et al.* 2011). Selenium was supplemented on 19/20 farms (Chambers and Laven 2026), but milking flock selenium status was unknown (not measured by the farmer) on 12/20 farms (Chambers and Laven 2026).

8.5.3 Research

We recommend four short-term research projects. (1) Milking hygiene: a prospective longitudinal study is indicated to confirm if wearing gloves during milking and applying post-milking teat disinfection (and for how much of the season it is applied) lower the risk of intramammary infection sufficiently to justify the labour and consumable resources under New Zealand conditions. Mandatory glove use (40% of farms) and all-season teat disinfection (also 40% of farms) were not common in our research, but neither practice was associated with subclinical mastitis (Chambers *et al.* 2026b). However, the low prevalence of subclinical mastitis and the small number of farms in the study may have limited our ability to confirm associations for farm-level factors like glove use and teat disinfection. Ewes' teats are often more laterally oriented than cows' (Chambers *et al*. 2025), which may alter the effectiveness of post-milking teat disinfection because the product can be displaced when the teats contact the legs. In dairy cows, Bartlett *et al*. (1992) visited farms monthly to collect mastitis, SCC, hygiene, environmental and mastitis-management data to investigate management associations with mastitis; this approach could be adapted for dairy sheep. (2) Rapid udder morphology scoring: a practical, rapid, standardised and repeatable system for scoring udder morphology should be developed and validated against the system used by Chambers *et al*. (2025). This would facilitate the application of selection for good conformation. Chambers *et al*. (2025) documented the morphology and prevalence of pathology of the teats and udders on dairy ewes on 20 farms but the technique, while robust, was too slow to apply at scale. Image-based or AI-assisted approaches to udder morphology scoring could be explored. Analogous work in dairy cows has shown that conformation traits

such as udder depth can be estimated automatically from 3D imaging systems, with moderate to high agreement with manual scoring, suggesting that similar approaches may be worth exploring in dairy sheep if validated against a robust manual reference method (Salau *et al*. 2017). (3) Multi-measure SCC and sampling strategy: collect repeated SCC measurements and milk cultures within and across lactations in a prospective cohort study. This would be used to (a) inform the minimum number of milk recording events that farmers are recommended to perform per season to manage mastitis; (b) update SCC thresholds when more than one SCC value is available; (c) determine the likelihood of self-cure over the dry period, which has implications for culling and the value of antibiotic dry ewe therapy; and (d) expand our understanding of the aetiology of intramammary infection in New Zealand dairy ewes. Despite Chambers *et al*. (2025b) collecting data from almost 900 ewes, the low prevalence of intramammary infection meant that only a small number of isolates was collected. In France, Berthelot *et al.* (2006) proposed a clear decision rule for diagnosing infected udders based on monthly SCC and bacterial culture data, illustrating the value of repeated SCC measurements for mastitis management. (4) The effect of freezing on milk culture: a split-sample, controlled laboratory evaluation of freezing and storage times on pathogen recovery from ewe milk is indicated to resolve "no growth" questions after culturing milk from ewes, such as that conducted by Smith *et al*. (2011). Previous research into clinical and subclinical mastitis on New Zealand farms revealed a high proportion of culture-negative samples (Chambers *et al.* 2024, 2026b), and both studies were limited by the unknown effect of freezing on the viability of bacteria in sheep's milk.

8.6 Medium term priorities (24-36 months)

8.6.1 Benchmarks

Update the benchmarks proposed above after collecting new data.

8.6.2 Practices

Produce short, sheep-specific SOPs, training modules and guidelines, informed by the four short-term research priorities. The bulk milk troubleshooting SOP can be refined, and industry recommendations can be made on the use of gloves and teat disinfection. Evidence-based guidelines can be produced for udder conformation scoring, SCC thresholds, the number and timing of milk-recording events to adequately manage mastitis, and handling of milk samples (including whether freezing is acceptable).

8.6.3 Research

We have identified 10 medium-term mastitis research priorities, grouped into four categories.

8.6.3.1 Treatments and vaccines

(1) Treatment of clinical mastitis: effective clinical mastitis treatments and milk withholding periods need to be found. Observational data and randomised controlled trials are of limited value given the low incidence (Chambers *et al.* 2024), so susceptibility testing of mastitis isolates would serve as a proxy and narrow the number of candidate products to take forward for further trial work needed for registration and to set expectations of their efficacy. This information could be augmented by the creation of a digital treatment and outcomes registry. (2) Vaccination: modelling the viability of vaccines for clinical and subclinical mastitis based on the prevalence and economics of New Zealand conditions is indicated. Such an approach was used for a vaccine against coliform mastitis in dairy cows (DeGraves and Fetrow 1991).

8.6.3.2 Risk factors

(3) Clinical mastitis: given its low incidence, a case-control study, run on farms with excellent animal records, is indicated to confirm clinical mastitis risk factors, such as indoors versus outdoors lambing. Waage and Vatn (2008) used a case-control methodology to identify mastitis risk factors for Norwegian sheep. (4) Subclinical mastitis: similarly, a case-

control study enrolling farms with high and low prevalences of subclinical mastitis would improve our understanding of subclinical mastitis risk factors given its low prevalence. (5) Lamb separation: on 14/20 farms in our study, lambs were mostly removed ≤10 days after birth and reared artificially, while on 6/20 farms most or all lambs were reared by the ewes and removed at a target age or weight (Chambers and Laven 2026). It is unclear if the timing of lamb separation affects the risk of mastitis, so a prospective cohort study of the impact of immediate versus delayed separation of lambs from ewes on udder health is indicated. In a randomised controlled trial of East Friesian ewes in the USA, McKusick *et al.* (2001) found no difference in post-weaning SCC between ewes whose lambs were removed at 24 h postpartum and machine milked, and ewes whose lambs remained with the ewe for 30 days postpartum before weaning and machine milking. However, clinical mastitis and bacteriology were not assessed, so the impact on mastitis per se remains unclear. (6) Udder conformation: longitudinal tracking of udder conformation on farms where selection is applied based on udder scoring is indicated to measure progress and determine associations with mastitis and milk quality. The scoring and selection program can be refined to develop a selection index for the ideal ewe for New Zealand conditions.

8.6.3.3 Farmer behaviours

(7) Timely detection of mastitis: qualitative research into how farmers identify mastitis may reveal practices that improve detection and identify common practices that limit detection. (8) Farmer drivers: qualitative research into economics and farmer decision-making would improve the uptake of best practices.

8.6.3.4 Surveillance and updating

(9) Characterisation of *Staphylococcus aureus*: genotypic characterisation of *S. aureus* isolates would be valuable for managing mastitis given the high proportion of clinical and subclinical mastitis cases that were caused by *S. aureus* (Chambers *et al.* 2024, 2026b).

Furthermore, many dairy sheep farmers also run dairy cow farms, which may be a source of staphylococci. As *S. aureus* is an important mastitis pathogen in both cattle and sheep, genotypic characterisation would help determine whether bovine and ovine isolates on mixed farms are unrelated or potentially shared. (10) Update the baseline: finally, the baseline work conducted in the 2022-2023 season should be updated following these initiatives and to reflect a more mature industry.

8.7 Call to action

Within 12 months, it is proposed that farmers and processors adopt sector targets: clinical mastitis incidence <1.5%, subclinical mastitis prevalence <2%, season mean bulk milk SCC <400,000 cells/mL and season bulk milk APC <5% consignments ≥100,000 CFU/mL. At the same time, farmers are counselled to implement routine milk recording, RMT use, ewe-level data collection, and a mastitis management protocol. Veterinarians are advised to reinforce supportive treatment while researchers, convened by an industry working group, should complete APC determinants studies and publish SOPs. By 36 months, updated targets and short, sheep-specific SOPs/guidelines will be in place (APC troubleshooting, hygiene, morphology scoring, SCC thresholds, sampling/handling). These will be underpinned by studies on gloves/teat disinfection, multi-SCC thresholds, freezing effects, clinical mastitis risk factors and treatments, subclinical mastitis risk factors, and *S. aureus* sources. Progress will be reviewed annually and funding aligned to benchmark gaps. These actions provide a practical framework for converting this evidence base into benefits for the New Zealand dairy sheep industry.

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9 SYNTHESIS

9.1 Introduction

As the final chapter of this thesis, the Synthesis recapitulates the research objectives, summarises the main findings and how they contribute to our knowledge of mastitis in New Zealand dairy sheep, outlines the strengths and limitations of the work, and makes final conclusions. It also briefly recaps the recommendations for future work made in Chapter 8.

Viewed as one piece of work, this research programme has set a national baseline for mastitis and milk quality on New Zealand dairy sheep farms. It starts with a foundation describing dairy sheep farms and teat and udder conformation and disease. Then it focuses on mastitis by defining its extent and features and the bacterial causes, and critical ewe and bulk milk quality indicators: somatic cell count, rapid mastitis test and aerobic plate count. Using the same baseline data, hypotheses relating to mastitis risk factors and associations between indicators are tested. Finally, all the findings are used to generate recommendations for the industry. In summary, an integrated evidence base has been established, from which industry benchmarks and management recommendations are derived. This is largely new information for New Zealand farmers, and it fills a gap in the literature that is centred on the northern hemisphere with its more intensive production models. Furthermore, it adds a counterpoint to more intensive industries on aetiology, burden of disease and risk factors under New Zealand conditions, and illustrates an industry in its formative stages rather than a mature industry. The latter point is of value to other countries starting their own dairy industries. These findings show how mastitis and milk quality behave in a relatively extensive, seasonal grazing system, providing a natural contrast to intensive and/or housed systems and helping clarify the importance of system-level factors such as housing and seasonality.

9.2 Main findings

Returning to the epidemiological triangle of host, agent and environment, several points have been revealed. Regarding the host, the ewes were generally younger than those in overseas research, comprised of a very diverse mixture of breeds (not predominantly one breed as is often the case in overseas industries), yet had udder conformation that was not dissimilar to that of ewes described overseas. They had little pathology, but, though rare, teat end hyperkeratosis was observed. Turning to the agent, Gram-positive bacteria, especially *Streptococcus uberis* and *Staphylococcus aureus* were the most common bacteria isolated. Staphylococci are well recognised as the predominant agents overseas, but *Streptococcus uberis* was prominent relative to published literature from overseas. Finally, the environment differed substantially to overseas, with seasonal lambing and a predominantly grazing (outdoor) management system across all the farms, but with clear variance between farms (and within farms) in many areas like lamb management. Lambing was almost entirely in the spring, which aligned with the peak clinical mastitis risk period. Milking machinery was modern but the milking hygiene practices of wearing gloves and disinfecting teats were not widely practiced. Three themes stand out.

9.2.1 Theme 1: Low incidence and prevalence of mastitis

It was unclear before this research project how common clinical and subclinical mastitis are in New Zealand dairy sheep. The incidence of clinical mastitis was 2.3%, which is low compared to the 10.0% (McDougall 1998) and 14.8% (Petrovski *et al.* 2009) incidences estimated for New Zealand dairy cows, but consistent with overseas estimates of 0.9% in Portugal (Queiroga 2017), and 1.7 cases per 100 ewe-months in Jordan (Lafi 2006). It should be noted, however, that while the incidence was low, clinical mastitis could be severe, with 25% of affected ewes having a fever and 26% having depression, and only 15% of ewes recovering without lasting sequelae. Most of the cases occurred around lambing time and

Streptococcus uberis, an environmental pathogen associated with udder contamination with dirt and faeces, was the single most common bacterial species to be isolated, suggesting that prevention should focus on the wet spring period. The high representation of *Streptococcus uberis* is unique to New Zealand, accounting for 14.0% of cases in the present study, while it accounted for 1.6% of cases in the study of Mork *et al.* (2007), was not found in the studies of Queiroga (2017) or Lafi *et al.* (1998), and only accounted for 1.9% of samples from non-dairy ewes with udder defects in the study of Ridler *et al.* (2021). A higher contribution by *Streptococcus uberis* to clinical mastitis for New Zealand dairy ewes, compared to dairy ewes in other countries, may be a feature of our predominantly outdoor, spring-lambing systems. In addition to focusing on the lambing period, given the already low incidence, efforts to manage clinical mastitis should seek ways to improve the probability of treatment success (with a full recovery and a functional gland), such as earlier detection, better treatments or the value of preventive vaccination. Qualitative research into how farmers identify mastitis may reveal practices that improve detection and identify common practices that limit detection.

The prevalence of subclinical mastitis was 6.4%, substantially lower than the 26% prevalence estimated by Greek researchers (Vasileiou *et al.* 2018) and the 34% prevalence estimated by las Heras *et al*. (1999) among Spanish ewes. Some of the difference 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 and/or a SCC >500,000 cells/mL in the present study) as well as altered neutrophil and lymphocyte proportions. The present study therefore in fact 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. Milk samples were frozen prior to culture in the present research, which may have altered bacterial

viability. The limited published research on the effect of freezing on culture of ewe milk suggests freezing reduces the viability of some bacterial species in milk, though this may be more pronounced in low CFU count samples (Smith *et al.* 2011), and deterioration appears to be greater for Gram-negative pathogens (Sanchez *et al.* 2003). Farm management and ewe demographics may also have played a role in the lower 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, and a lower prevalence of subclinical mastitis (overall and staphylococcal) among extensively managed flocks. The ewes in our study had a median age of 2 years, possibly because more than half of the ewes were on farms in the first 2-3 seasons of production. The age of ewes in other published studies has 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 0-11.4% among first and second parity ewes across the three visits, but 5.5-22.2% for third and greater parity ewes. The lower prevalence in the present study may reflect the younger age of the ewes, but age was not included in the final model because age data were only available for approximately 75% of ewes. Therefore, younger age contributing to the lower prevalence remains a hypothesis only and requires follow up work to confirm. This research supports the notion that the prevalence of subclinical mastitis is lower in grazing, machine milked dairy sheep, and the New Zealand industry should aim to preserve this advantage, especially if it intensifies and experiences an increase in prevalence that approaches what is reported overseas.

Paradoxically, while the prevalence of subclinical mastitis was relatively low in the 20 flocks in this study, ewe SCCs were relatively high compared to the limited published information

from overseas. The geometric mean SCC was 169,039 cells/mL, lower than the geometric mean of 539,000 cells/mL in a previous study of New Zealand dairy sheep (McDougall *et al.* 2001), but substantially higher than the geometric means of 72,444 (Ariznabarreta *et al.* 2002) and 95,499 (Gonzalo *et al.* 2002) cells/mL among culture-negative Spanish ewes. The reasons are unclear but may reflect different management systems (including monthly milk recording in one of the Spanish studies), a possible effect of freezing on sample viability (freezing of samples in the New Zealand study may have caused some false negative bacteriological diagnoses and therefore inflated the SCC of culture-negative samples), or selection pressure towards ewes with lower SCCs overseas. This discrepancy is important because it supports the concern about false negative bacterial culture results but also suggests the method of milk sampling for SCC (whole milk fraction as in milk recording or foremilk as in the present research project) should be explored because of potential implications on SCC estimation. Follow up work should compare SCC and milk handling methodologies and investigate potential genotypic differences in SCC.

The aetiology of subclinical mastitis was dominated by staphylococci, consistent with the aetiology reported in overseas industries (Fthenakis 1994; Vasileiou *et al.* 2018; Michael *et al.* 2023). Bulk milk SCC was also in the mid-range of reports from overseas. The geometric mean of 659,491 cells/mL was higher than the geometric mean of 488,000 cells/mL reported by Lianou *et al*. (2021) in Greece but lower than the 1,072,000 cells/mL reported by Gonzalo *et al*. (2019) and 1,093,000 cells/mL reported by de Garnica *et al*. (2013) in Spain.

On one hand, New Zealand conditions may be conducive to lower mastitis burdens, and there is some evidence from overseas to support the notion that the prevalence of subclinical mastitis is lower in more extensive systems. On the other hand, it is possible that the true incidence of clinical mastitis and prevalence of subclinical mastitis were underestimated due

to methodological limitations, namely reliance on farmer diagnosis of clinical mastitis and the possible reduction in bacterial viability caused by freezing and storing milk samples.

In summary, it can be concluded that the New Zealand dairy sheep industry is fortunate to have low incidence of clinical mastitis that is on par with the limited data from overseas, and a low prevalence of subclinical mastitis that is substantially lower than reports from overseas. What remains uncertain is whether the true prevalence of subclinical mastitis was underestimated due to freezing of milk samples. For now, the focus should be on maintaining the low incidence/prevalence, preparing for managing increases or spikes in the prevalence, and validating the low prevalence of subclinical mastitis by further sampling and investigating the effect of different milk handling practices on bacterial culture.

9.2.2 Theme 2: There is a relationship between aerobic plate count and mastitis

One of the most common requests from farmers and processors when developing this research program was to help them resolve the challenge of high bulk milk APC. Chapter 6 presents the first known research to find an association between mastitis and APC at the ewe level. While this does not mean that mastitis is necessary to cause high bulk milk APC, it does mean it may sometimes be sufficient (or at least cannot be eliminated as a potential cause). This aligns with the anecdotal observations of farmers who have managed high bulk milk APC by performing an RMT on some or all ewes in the milking flock and removing the ewes with the highest RMT scores. Whether the drop in APC that follows was caused by removing those ewes from supply is unknown, but the work here means it at least cannot be ruled out.

However, while intramammary infection and/or mastitis were associated with higher odds of elevated APC, they did not have strong predictive power because the prevalence of mastitis was low in the study population. Although ewes with high CMT scores had substantially

higher odds of elevated APC, the sensitivity of CMT was low, identifying only 41% of affected ewes. In addition, the low prevalence of both elevated APC and high CMT scores resulted in a low positive predictive value. Consequently, many ewes with elevated APC would remain undetected, and removing ewes based on CMT alone would be an inefficient strategy for reducing bulk milk APC. Careful messaging to farmers is necessary to ensure they rule out other more routine causes of high bulk milk APC, such as poor milking and plant hygiene or refrigeration, before trying to identify individual ewes that may be driving the high APC.

Regarding bulk milk, by using more robust statistical methods than have been used previously, this study has shown that SCC has a weak association with APC but poor predictive power for high APC, so it should not be used as a tool to manage high bulk milk APC. An association between mastitis and APC has been shown at the ewe level, but the predictive power was low, and more work is needed to confirm the causality and the extent to which mastitis contributes to high bulk milk APC overall.

9.2.3 Theme 3: Improving the diagnosis and management of mastitis

The main purpose of this research was to establish a baseline for New Zealand dairy ewes to inform work aiming to make improvements. Many clear steps for improvement have now been identified.

9.2.3.1 Farm data limitations

The frequent lack of ewe-level data are a significant limitation to progress. Many farmers manage ewes as a mob and therefore do not maintain ewe-level data such as age and lambing date. Routine milk recording was only performed on 7/20 (35%) farms. The 65% of farms without milk recording data therefore have no ewe-level SCC information and cannot easily identify ewes at higher risk of having mastitis. A priority for the New Zealand industry is to

support and possibly incentivise farmers to perform at least one milk recording event so they can more easily manage mastitis, as well as rank ewes on productivity and develop farm data management systems.

9.2.3.2 Diagnostic tools

The diagnostic values of SCC and RMT were established. A SCC threshold of approximately 400,000 cells/mL had the greatest accuracy for diagnosing intramammary infection (IMI) using a single SCC measurement, but while it had a specificity of 0.88, its sensitivity was only 0.64. This means that SCC has a high negative predictive value (low-SCC ewes are highly likely to be uninfected), but high-SCC ewes are still more likely to be uninfected than infected. At least one routine milk recording event with SCC measurement per season on dairy sheep farms is suggested to identify ewes with subclinical mastitis, but farmers should not make decisions on the basis of a single high SCC. Follow-up SCC, RMT or culture are advised to confirm the ewe's infection status. Using multiple SCC measurements to determine mastitis status has been recommended (Berthelot *et al.* 2006). Therefore, local research is required to determine whether the same threshold should be applied when there is more than one SCC measurement within a lactation, and what the minimum number of SCC measurements should be. Collecting repeated SCC measurements and milk cultures within and across lactations in a prospective cohort study would inform the minimum number of milk recording events that farmers are recommended to perform per season to manage mastitis, update SCC thresholds, determine the likelihood of self-cure over the dry period, and expand our understanding of the aetiology of intramammary infection in New Zealand dairy ewes. Despite collecting data from almost 900 ewes, the low prevalence of intramammary infection meant that only a small number of isolates was collected (Chapter 5).

Mean $\log_{10}$ SCC increased linearly with RMT score but its agreement was only moderate (Kendall's tau = 0.47). Users should understand that negative and very high RMT scores reliably represent opposite ends of the SCC spectrum, but that there is some overlap between each of the scores (0, trace, 1, 2 and 3) due to the subjective and imprecise nature of RMT. This underscores the value of SCC data. The RMT can be promoted to screen ewes at key points for mastitis, such as the transition from the colostrum flock to the milking flock. The evidence also supports the use of the RMT to confirm suspected mastitis cases based on visual signs, due to the possible over-diagnosis in farmer-reported clinical mastitis (Chapter 4).

Milk culture is valuable for understanding the epidemiology of mastitis on a given farm. Nearly half of all the clinical mastitis milk samples were culture negative (i.e., no bacteria were grown) (Chapter 4), and the SCCs of the culture-negative randomly sampled ewes in Chapters 5 and 6 were higher than those reported for culture-negative ewes overseas. In both the clinical and subclinical mastitis arms of this study, milk samples were frozen before culture. Previous work has suggested that freezing can reduce the viability of mastitis pathogens (Smith *et al.* 2011), though it can also increase the recovery rate of some pathogens (Schukken *et al.* 1989; Sanchez *et al.* 2003). A clear learning from this study is that the impact of freezing on the culture of sheep milk samples must be determined. A split-sample, controlled laboratory evaluation of freezing and storage times on pathogen recovery from ewe milk is indicated to resolve "no growth" questions after culturing milk from ewes. In addition, the use of molecular methods like pathogen PCR could help differentiate low sensitivity from true negative culture results.

In the meantime, establishing an industry protocol for identifying and managing ewes with mastitis would help farmers manage mastitis by giving them a process to follow. For example, a sequence of using SCC and/or RMT to detect and confirm elevated SCC,

followed by targeted milk culture and monitoring of high-SCC ewes. This would then be updated when the recommended future research is completed.

9.2.3.3 Management

With a median of four seasons since commencing production (including the season of the study) across the 20 farms in the study, the industry is young and therefore in the early stages of a journey of exploration and consolidation. Chapter 2 revealed that there is significant variation not only between farms, but also within farms in one season in many farm practices, most notably lamb management and the window of milk harvesting. Indeed, the only common features we identified were spring lambing, employing a predominantly outdoor management, selenium supplementation, and clostridial vaccination. Many farm practices were dictated by resource availability. For example, the timing of lamb separation was often a function of whether there was space in the lamb rearing shed or there were enough staff to rear the lambs. This means that advisors should learn about each farmer's goals and constraints before offering mastitis management advice because of practical constraints. Furthermore, recommendations may need to be changed during the season if management systems are updated.

In Chapter 8, targets were proposed for clinical mastitis incidence (<1.5%), subclinical mastitis prevalence (<2%) and bulk milk quality (<5% of bulk milk consignments exceeding 100,000 CFU/mL for APC and season mean SCC <400,000 cells/mL). These evidence-based targets provide clarity to the industry and are clearly achievable because they are the means of the best 25% of the farms enrolled in this study.

The farm descriptive data revealed that teat disinfection and wearing of gloves during milking were not routinely performed on the majority of farms. Neither practice was found to

be associated with subclinical mastitis, but that may have been limited by the low prevalence. This work has identified the need to confirm the value of these practices.

Now that the incidence and prevalence of clinical and subclinical mastitis, respectively, are known, and the bacterial causes have been identified, it is possible to explore the economic viability of vaccination. Modelling can be used to determine the net benefit of vaccines based on expected efficacy compared to the costs.

Together, these three themes converge on a small set of thesis-wide advances, summarised below.

9.3 Contribution to knowledge

What we now know overall: New Zealand dairy sheep mastitis is low-frequency but high-impact, with clinical cases concentrated around lambing and showing severe outcomes; this shifts prevention priority to early-lactation environmental control. Subclinical mastitis prevalence is low in New Zealand's extensive, machine-milked systems, suggesting a structural advantage worth protecting as the industry matures. Returning to the epidemiological triangle, should teat and udder conformation change over time due to selection (host), stocking density increase and housing become more common (environment) or mastitis pathogens shift towards more virulent staphylococci (agent), the prevalence of subclinical mastitis may approach what is reported in more intensive industries overseas. APC problems cannot be understood without considering mastitis, but the relationship between SCC and APC at the bulk milk level is too weak for SCC to serve as a management proxy. Current surveillance and decision-making are constrained by data, making milk recording and repeat SCC/RMT protocols the most immediate area to develop on farm.

Specifically, this body of research has added the following knowledge to the scientific literature:

- First concrete estimates of the extent of clinical and subclinical mastitis in New Zealand dairy ewes,
- Quantitative data on the timing, severity, treatment and outcomes of clinical mastitis,
- New Zealand-specific data on the bacterial causes of mastitis (including dominance of *Streptococcus uberis* in clinical mastitis),
- Subclinical mastitis risk factors,
- The diagnostic accuracy of somatic cell count and the rapid mastitis test for diagnosing intramammary infection,
- Proven association between high APC of composite ewe milk samples and mastitis and weak link between bulk milk somatic cell count and bulk milk aerobic plate count,
- Benchmarks from the top 25% of farms,
- A list of practices and research priorities to build upon this baseline research project,
- Features of New Zealand dairy sheep farms (size, staffing, management, productivity, milk harvesting practices, animal health practices),
- A baseline description of teat and udder conformation on multiple farms, and the between-farm variation,
- Awareness of the low prevalence of teat and udder pathology on New Zealand farms.

9.4 Overall strengths and limitations

A major strength of this research is its external validity. Data were collected from almost 900 ewes on 20 farms, representing approximately 50% of commercial farms in operation at the time. The farms were purposively selected to represent a wide range of farming systems in New Zealand, while being managed by farmers who were willing to participate. The conclusions are therefore more generalisable than data collected from a single farm or farms

that are more accessible or better set up for research. The impact of enrolling a different set of 20 farms is unclear without repeating the study. However, recruiting different farms would be limited by farmer compliance with the study protocols and potentially of worse internal validity.

Recruiting more farms would have improved the study's estimates of true population statistics. Infectious diseases are often clustered, meaning animals within a farm are more alike than animals on different farms. This phenomenon acts to reduce the effective sample size because ewes within farms are not fully independent, which means that each additional ewe enrolled adds less independent information than the previous ewe. Using the same sample size calculation as detailed in Chapter 5, if the same number of ewes (n=15) were recruited from 30 farms instead of 20 farms, the precision (half the width of the confidence interval) would have tightened from 0.068 (20 farms) to 0.055 (30 farms). It was decided that the small amount of extra precision was outweighed by the costs imposed by adding more farms, especially for a first of its kind baseline study. Ultimately, the prevalence was estimated to be 6.4% with a 95% CI of 4.6-8.7%, a satisfactorily narrow CI. Additionally, the intraclass correlation coefficients (ICC) for clinical and subclinical mastitis were 0.017 and 0.04, respectively. These values mean that only a small proportion (1.7% and 4%, respectively) of the variation in clinical and subclinical mastitis was explained at the farm level, further reducing the value of adding more farms. In practice, this means the prevalence estimate is already reasonably stable for a first national baseline.

While a large proportion of farms was represented, only approximately 15 ewes were enrolled at each visit for the randomly selected ewe arm of the research. A larger number would improve the study's precision/power and potentially the internal validity, by recruiting a wider range of ewes with different demographics (such as age) and/or allowing more subgroups to be compared. The number of ewes enrolled per farm was based on a two-stage

cluster sampling calculation, which was made to optimise the precision of the estimated prevalence of subclinical mastitis. Because subclinical mastitis was expected to be clustered within farms, each additional ewe enrolled adds less independent information than the previous ewe. Furthermore, enrolling more ewes increases the time needed to complete each visit. Initially, 30 ewes were enrolled but this was reduced to 15 ewes after the first few farm visits because of concerns about how long the process took and therefore how long the ewes were off feed. Again, using the same formula as in Chapter 5, reducing the number of ewes per farm (with n=20 farms) from 30 to 15 ewes only changed the precision from 0.059 to 0.068, so a decision was made to prioritise animal welfare and farmer engagement over precision. Future studies could focus on a narrower set of measurements and afford to enrol more ewes per farm or increase the number of operators to speed the process up.

Collecting data from a smaller number of highly compliant farms with excellent records and data collection would have increased the internal validity of this research. Farmer compliance was relied on (Chapter 4) and data were missing or based on farmer recall on some farms, and the scale of the study meant that milk samples had to be frozen before culture. Farmer data capture introduces the risk of random error, but also potentially systematic error if farmers tended to over- or underestimate some parameters (the number of cases of clinical mastitis that were not entered into the app, for example). These threaten the internal validity, but a deliberate choice was made to optimise for external validity given the overarching aim to provide baseline data. However, this study aimed to establish a broad baseline, and future work can be refined to mitigate such limitations. Furthermore, for clinical mastitis (which required farmer reporting of cases), two sets of incidence estimates were presented: incidence based on the cases that were reported during the trial, and incidence based on those cases and any others that the farmers knew or suspected were not reported. The two estimates did not

differ substantially, suggesting that the low incidence of clinical mastitis reported in this study is likely to be accurate.

Despite some limitations with internal validity, consistent on-farm and laboratory methods were applied and robust statistical analyses that withstood peer review were used, providing confidence in the conclusions that have been made in spite of some of the limitations. The scene is now set to pursue specific hypotheses on a restricted set of farms to optimise for internal validity. This is another strength of the study: by collecting a wide range of baseline data, many second phase research priorities have become clear. Beforehand, it was difficult to identify those priorities.

The data were only collected over a single season. The present work is unable to describe the between-season stability of its estimates, and it should be updated. Variation in climate, changing management trends, and random variation could cause the extent and characteristics of mastitis to change across time.

The freezing of milk samples stands out as a significant open question as it had an unknown effect on the milk culture results. If a significant proportion of “no growth” samples were false negatives, the prevalence of subclinical mastitis would have been underestimated. Furthermore, the data on the bacterial causes of both clinical and subclinical mastitis would have had a higher resolution due to there being more bacteriologically positive samples to work with. The relative proportion of each bacterial species or genus may have been biased if some species or genera were more affected by freezing than others, as has been suggested in other work. Practically speaking, shipping samples immediately would have substantially increased the costs of the study and created resource bottlenecks when collecting large numbers of samples on the same day and was therefore not feasible. This work has highlighted the need to test the effect of freezing on sheep milk sample culture. Doing so

would pressure test the findings of this work and, if a significant effect of freezing is found, confirm that further research without freezing of samples is required.

While the low incidence of clinical mastitis and low prevalence of subclinical mastitis were positive findings, they also meant that a smaller number of mastitis pathogens were cultured, limiting the precision of our understanding of the aetiology of mastitis. In Chapter 5, only 10 *Staphylococcus aureus* isolates were found at the gland level. To better understand the aetiology of mastitis, especially subclinical mastitis and the genotypic profile of staphylococci, future work should focus on high-SCC ewes, among which the prevalence of intramammary infection will be higher.

Risk factor analysis was limited by the lack of ewe-level data on many farms. Ideally, a case-control study would have been run to identify clinical mastitis risk factors, but the resolution of ewe-level data was so poor on many farms that risk factors like ewe age, litter size at lambing, and date of lambing could not be explored.

The accuracy of SCC was based on a single measurement. It is likely that multiple measurements will improve the accuracy of SCC at diagnosing intramammary infection. Further work is needed to explore this.

Finally, this research includes a chapter that takes the findings and makes recommendations for the New Zealand industry. This is a strength because it moves from describing reality to applying the findings in a practical way, for the benefit of the industry and future researchers.

In summary, the research programme was limited by the practicalities of running trials on commercial farms (missing data due to limited records and reliance on recall), time and resource constraints that placed an upper bound on the number of farms and ewes that were enrolled, potential false negative milk culture results due to freezing of milk samples, and running the trial over one season without repeated milk quality measurements. These factors

may have introduced bias: the prevalence of subclinical mastitis could have been underestimated, whereas the incidence of clinical mastitis could have been either over- or underestimated due to potential false positive diagnoses. False negative cultures may have differentially affected certain bacterial groups, distorting the true breakdown of bacterial species. These limitations were inherent to the study design and were known trade-offs that were made to achieve a study of this scale. They provide clear guidance for future research designs. The programme's main strengths are its large scale and external validity, the foundational baseline information that was collected in a consistent and systematic fashion, and the practical recommendations that have come from it.

9.5 Final conclusions

Each objective is summarised in detail below but, overall, New Zealand dairy sheep mastitis is currently a low-incidence/prevalence but high-impact condition, with management and surveillance constrained by limited records and screening. The low prevalence and modest isolate numbers mean that causal inference and ranking of intervention priorities remain premature, reinforcing the need for a second phase of surveillance and hypothesis-generating work. Based on what we have learned, future research can be prioritised to address gaps, particularly in relation to causality, risk factors and diagnosis, which our largely descriptive work was not designed to fully support. The immediate priorities (Chapter 8) are therefore framed to improve data quality and decision making before more complex objectives are pursued in the short and medium term. Together, these seven objectives provide the first comprehensive baseline on mastitis and milk quality in New Zealand dairy sheep, contribute a novel pastoral-system perspective to the international literature, and establish a clear platform for targeted intervention and further research.

9.5.1 Objective 1 (descriptive analysis of New Zealand sheep milking farms)

The typical dairy sheep farm was in its fourth season of operation at the time of the study. Farm practices varied widely, particularly in lamb removal timing and milk-harvesting period. Automation was partial (in-shed feeding was common, automatic cup removers were present on one-third of farms, and no farms had automatic teat sprayers). Common features were spring lambing, predominantly outdoor management, selenium supplementation, and clostridial vaccination. Milk recording and ewe data were limited.

9.5.2 Objective 2 (characterise udder and teat conformation and health)

Udder morphology was like that observed in overseas dairy sheep. Teat dimensions, udder depth, separation, suspension, and teat placement, and presence of supernumerary teats varied between farms. Udder depth, separation and suspension scores decreased with age, while teat placement score and the prevalence of asymmetry increased with age. Teat and udder pathology was rare.

9.5.3 Objective 3 (determine the incidence and describe the aetiology of clinical mastitis and describe how farmers treat clinical mastitis and the outcomes)

Clinical mastitis was diagnosed in 0-6% of ewes at the farm level, with an overall incidence of 2.3%, consistent with overseas reports. Cases occurred mostly in early lactation. Fever was diagnosed in 25% of cases and depression in 26% of cases, showing that clinical mastitis can be severe. It is also challenging to resolve. Treatment was given to 46% of cases, with tylosin being the most commonly used treatment (50% of treated cases). Only 15% of ewes made a recovery without sequelae. Nearly half of all the milk samples submitted were culture negative. *Streptococcus uberis*, non-aureus staphylococci, and *Staphylococcus aureus* were the most common isolates.

9.5.4 Objective 4 (determine the prevalence, describe the aetiology and identify risk factors for subclinical mastitis)

The prevalence of subclinical mastitis was 6.4%. The most common pathogens were non-aureus staphylococci and *S. aureus*. 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 prevalence was low compared to studies of more intensively farmed ewes in the northern hemisphere, but the bacterial causes were consistent.

9.5.5 Objective 5 (describe ewe-level somatic cell count and aerobic plate count and their associations with intramammary infection, determine the correlation between the rapid mastitis test and somatic cell count, find somatic cell count thresholds for diagnosing intramammary infection, and identify risk factors for elevated ewe-level aerobic plate count)

The geometric mean somatic cell count of randomly selected ewes was 169,039 cells/mL, varying between farms and decreasing across visits, and relatively high compared to the limited reports from overseas. Mean $\log_{10}$ SCC increased linearly with RMT score but the correlation between the ewe's highest gland-level RMT score and SCC was moderate (Kendall's tau = 0.47). A SCC threshold of ~400,000 cells/mL had the greatest Youden's index for diagnosing intramammary infection using a single SCC measurement but was limited by a low positive predictive value due to the low prevalence of infection. Elevated RMT score and SCC, positive milk culture, and subclinical mastitis were identified as risk factors for elevated APC.

9.5.6 Objective 6 (describe the distributions of bulk tank somatic cell count, aerobic plate count, and determine their association)

Bulk milk SCC had a geometric mean of 659,491 cells/mL, with farm geometric means ranging from 358,198 to 1,278,382 cells/mL. Aerobic plate count exceeded 100,000 CFU/mL in 22.3% of consignments, peaked in August, and the farm-level proportion exceeding this threshold varied from 2.9% to 100%. A doubling in SCC was associated with approximately double the odds of APC exceeding 100,000 CFU/mL, but high APC was not well predicted by SCC. Farm-level aspirational targets were proposed for SCC and APC.

9.5.7 Objective 7 (generate a narrative review of the research and recommend benchmarks, practices and research priorities for the future)

Immediate, short-term, and medium-term priorities for future actions were proposed based on the findings addressed by objectives 1-6. Benchmarks for clinical mastitis incidence (<1.5%, Chapter 4), subclinical mastitis prevalence (<2%, Chapter 5), and bulk milk aerobic plate count (<5% of consignments ≥100,000 CFU/mL, Chapter 7) and somatic cell count (season mean <400,000 cells/mL, Chapter 7) were set using the mean of the best 25% (best quarter) of farms (Chapter 8). On-farm practices most likely to achieve these benchmarks were summarised, including milk recording (Chapter 2), use of the rapid mastitis test (Chapter 6), somatic cell count thresholds (Chapter 6), milking hygiene methods (Chapter 2) and udder conformation scoring (Chapter 3). Research priorities included determining the factors associated with high bulk milk aerobic plate count (Chapters 6 and 7), determining the effect of freezing on sheep milk sample culture result (Chapter 4-6), clarifying mastitis identification and management when multiple milk recording events take place within a season (Chapter 6), extending the preliminary investigation of mastitis risk factors and treatments (Chapters 4 and 5), confirming the value of milking hygiene practices (Chapters 2 and 6), and developing a rapid udder conformation scoring system (Chapter 3).

There are several pathways for future research that follow on from this thesis. Future research should prioritise questions that most affect the interpretation and practical application of these baseline findings. First, because controlling high bulk milk aerobic plate count is a priority for the industry, more advanced work on the causal relationship between mastitis and elevated bulk milk aerobic plate count is needed. Second, validity work is necessary to strengthen inferences, particularly quantifying the effect of freezing sheep milk samples on bacterial culture results (subclinical mastitis prevalence and diagnostic test accuracy and thresholds) and converting the single-measurement thresholds for somatic cell count to repeated-measure thresholds. Third, targeted culture of high-somatic cell count ewes is needed to augment the small aetiology dataset and improve the precision of risk factor inference due to the different predisposing conditions for each pathogen. Finally, once these foundations are strengthened, qualitative and interventional studies can be more efficiently developed, including evaluating the value of milking hygiene practices.

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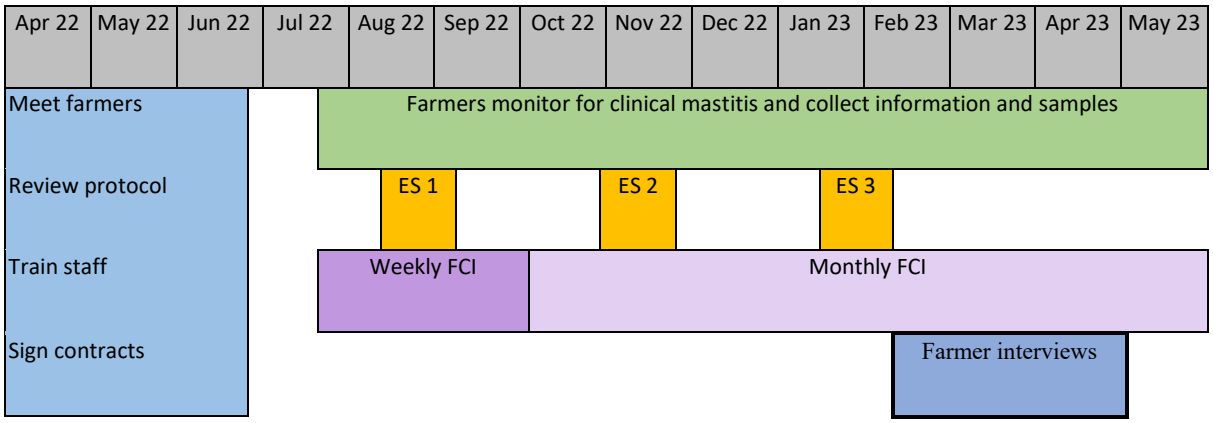
10 APPENDIX – STUDY PROCEDURES

This section contains parts of the study protocol that describe how farms were identified and recruited, farmer and technician training, and sample collection, handling and processing up to the point where the samples were sent for testing.

It contains a chronological summary of events (10.1), recruitment of farms (10.2), study procedures (10.3), information on the smartphone application developed for farmers to collect clinical mastitis information (10.4), farmer and study operator guides (10.5), data capture forms (10.6), the standard operating procedure (SOP) for collecting milk samples (10.7), and references (10.8).

These materials often use the future tense because they were written prior to the study commencing.

10.1 Summary of events



ES Ewe sampling visit
FCI Farmer check in (to ensure compliance with clinical mastitis recording and sampling)

10.2 Recruitment of farms

The aim was to recruit a mixture of farms (including some who supplied processors and others who processed their own milk) across different regions. Farms were convenience selected based on these criteria, and also the willingness of farmers to participate and comply with the study procedures, provide access to flock and sheep level demographic data, and share flock test records (for those farms that conduct routine flock testing, i.e., collection of milk samples for measurement of milk quantity and quality parameters including somatic cell count). Farmers who supplied the three processors (Maui Sheep Milk, Spring Sheep and Sheep Milk NZ) were recruited by consulting the processors, who in turn engaged their farmers and provided lists of farmers to recruit.

All participating farmers completed an owner consent form (10.2.1)

10.2.1 Owner consent form

Thank you for participating in the study "Establishing baseline mastitis information on commercial New Zealand sheep milking farms".

Please read the following information and consent declaration carefully. If anything remains unclear, please ask us.

Owner's name:	
Manager's name (if applicable):	
Farm address:	
Postcode:	
Owner/manager phone number:	
Approximate number of milking ewes in the 2022/2023 season:	

- I hereby certify that I am the owner (or fully authorized representative) of the above animals, and that I am at least 18 years of age.
- I voluntarily allow the animals under my care to participate in this study.
- I will allow the Investigator to access my property daily for the scheduled visits.
- I will provide or allow the Investigator access to mastitis and milk quality data, including (when applicable) bulk milk data, farm records, and herd test data.
- During this study, I will immediately inform the Investigator or Attending Veterinarian of any unusual or unexpected occurrences, whether or not I feel that the development is study related.
- I will inform the Investigator or Technicians of any occupational safety hazards on my property.
- I understand that the Investigator and associated personnel will treat my information as highly confidential and it will only be shared publicly in after being anonymized and combined with information from other participating farms.
- I understand that results and other study information relating to my animals may not be available to me immediately.
- I have read and fully understand the animal owner informed consent. The Investigator has explained all aspects and risks of the study. The Investigator or Study Veterinarian has satisfactorily answered all my questions.

Owner Signature: _____ Date: _____

Investigator Signature: _____ Date: _____

10.3 Study procedures

10.3.1 Clinical mastitis

Chronological order of activities and relevant protocol sections and forms:

Date	ACTIVITIES	RELEVANT SECTIONS	RESPONSIBLE PERSON
April-May 2022	Meet farmers to review protocol, train staff in mastitis detection and milk sampling, and sign contracts	10.2 10.3.1 10.4 10.5.1 10.6.1	Greg Chambers
July 2022	Examine ewes at lambing or first milking for clinical mastitis	10.3.1	Farm manager
	Collect clinical mastitis case data and samples	10.4 10.5.1 10.6.1 10.6.1	Farm manager
	Contact farmers weekly to drive clinical mastitis objective		Sarah Hurst/Greg Chambers
August 2022	Examine ewes at lambing or first milking for clinical mastitis	10.3.1	Farm manager
	Collect clinical mastitis case data and samples	10.4 10.5.1 10.6.1 10.6.1	Farm manager
	Contact farmers weekly to drive clinical mastitis objective		Sarah Hurst/Greg Chambers
	Collect ewe samples	10.3.1	Sarah Hurst
September 2022	Examine ewes at lambing or first milking for clinical mastitis	10.3.1	Farm manager
	Collect clinical mastitis case data and samples	10.4 10.5.1 10.6.1 10.6.1	Farm manager
	Contact farmers weekly to drive clinical mastitis objective		Sarah Hurst/Greg Chambers
October 2022	Examine ewes at lambing or first milking for clinical mastitis	10.3.1	Farm manager
	Collect clinical mastitis case data and samples	10.4 10.5.1 10.6.1 10.6.1	Farm manager
	Contact farmers monthly to drive clinical mastitis objective		Sarah Hurst/Greg Chambers
November 2022	Collect clinical mastitis case data and samples	10.4 10.5.1 10.6.1 10.6.1	Farm manager
	Contact farmers monthly to drive clinical mastitis objective		Sarah Hurst/Greg Chambers
	Collect ewe samples	10.3.1	Sarah Hurst
December 2022 – January 2023	Collect clinical mastitis case data and samples	10.4 10.5.1 10.6.1	Farm manager

		10.6.1	
	Contact farmers monthly to drive clinical mastitis objective		Sarah Hurst/Greg Chambers
February 2023	Collect clinical mastitis case data and samples	10.4 10.5.1 10.6.1 10.6.1	Farm manager
	Contact farmers monthly to drive clinical mastitis objective		Sarah Hurst/Greg Chambers
	Collect ewe samples	10.3.1	Sarah Hurst
March – May 2023	Collect clinical mastitis case data and samples	10.4 10.5.1 10.6.1 10.6.1	Farm manager
	Contact farmers monthly to drive clinical mastitis objective		Sarah Hurst/Greg Chambers

10.3.1.1 On farm procedures

Prior to start of lambing in 2022, farmers will be provided with a mastitis kit and protocol (appendix 10.4) that:

a) Defines clinical mastitis

b) Lists the information to be collected and procedures to be conducted

c) States what to do with the information

d) Describes the sterile milk sample collection SOP

e) An equipment checklist for the mastitis kit

All staff who may detect clinical mastitis on each farm will be trained on the protocol to ensure a consistent method across all farms. The farm manager will be responsible for ensuring new staff receive induction training on mastitis detection. A three-minute video on how to collect a sterile milk sample is available on YouTube at https://youtu.be/KkmF0IFlAHI.

Clinical mastitis will be defined as:

- A change in the appearance of milk (e.g., clots, blood, watery milk, red milk), AND/OR
- Signs of inflammation in the gland (swelling, uneven udder, pain, lumps, discharging sores)

Events **not** considered to be clinical mastitis:

- Positive RMT without clinical signs
- Blood in the milk without inflammation (e.g., swollen, hot or painful gland)
- Conditions limited to the skin, such as orf, will be excluded.

All ewes will be manually examined for clinical mastitis at the first time they are handled after lambing (e.g., at lambing or at first milking).

When a case of clinical mastitis is detected, the following procedures will occur:

- Using the dairy sheep mastitis app, or a laminated printout of Data Collection Form 1, farm staff will collect detailed ewe and mastitis information.
- A duplicate 10 ml milk sample will be collected by the farmer or worker using aseptic technique and frozen on farm at or below -20°C.
- The ewe will be treated according to farm plan (no guidance will be provided)
- Information will be updated on the form as the case progresses.
- Frozen samples will be picked up by the EpiVets team and contractors as they visit the farm for the randomly selected ewe events and transported on ice back to the clinics. The first sample will be shipped to Massey University for frozen storage until microbiology is performed, using insulated boxes with ice, and packed so the vials are

stable and remain upright. The second (duplicate) sample will be retained at EpiVets or VetEnt Ashburton to spread the risk of freezer failure.

The farmers have adhesive labels in their mastitis kits with fields for date, farm, ewe ID, gland, person name/initials. They will also write the ewe's ID in marker pen on the vial lid in case labels come off. NB it was assumed to be impractical for a veterinarian to sample mastitis cases due logistics.

Farm demographic and management information will be collected through an interview in 2023. This does not need to be collected for every case as it applies to the farm overall.

10.3.1.2 Laboratory procedures

Frozen samples will be thawed at Massey University for microbiology. Microbiology will be restricted to bacterial causes of mastitis. Contagious agalactia (caused by mycoplasma) will not be covered in this study. However, milk samples will be frozen and retained, should there be interest in mycoplasma.

Thawed samples will be swirled and 10 µL of milk aseptically collected and deposited as a drop on a quarter of a 5% sheep blood agar plate (Fort Richard; Auckland, NZ) and spread using a sterile spreader. Plates will be incubated aerobically at 35–37°C for 40–48 hours. After incubation, plates will be inspected for bacterial growth and the number of colony types recorded. Plates with three or more colony types will be defined as contaminated and not analysed further. For samples with one or two colony types, the number of colonies will be recorded for each type. A minimum of one colony type with three or more colonies will be necessary for the plate to progress to the next stage, except where a colony morphologically resembles *Staphylococcus aureus*, when only one colony will be required (Gonzalo *et al*.

2002). When all colony types have <3 colonies (and none resemble *Staph. aureus*), the sample will be classified as "no growth", and no further action taken. If a colony type had ≥3 colonies (or resembled *Staph. aureus*), one isolated colony will be picked and sub-cultured onto a new 5% sheep blood agar plate and incubated as above to generate an isolate. The isolates will be submitted for matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF; Microflex LT Biotyper; Bruker Daltonics, Billerica, MA, USA) for bacterial identification at mEpiLab, Massey University. A protein extraction protocol will be used, in which 1 µL of bacteria will be added to 300 µL of high-performance liquid chromatography-grade water, mixed in 900 µL of ethanol with a vortex mixer, centrifuged, and the pellet dried.

For cases where the original culture is defined as contaminated, or isolates are not identifiable by MALDI-TOF, the secondary sample will be thawed and cultured using the same procedure. In such cases, the result from the secondary sample will be used in the analysis.

The bacteriology results will be categorised into six groups: (1) growth of *Streptococcus uberis*; (2) growth of *Staph. aureus*; (3) growth of non-aureus staphylococci (NAS); (4) growth of "other" bacteria; (4) no growth or unidentifiable bacterium; (5) mixed growth (two colony types); and (6) contaminated samples.

Initially, a conservative value of ≥ 10 colonies was considered as the threshold for a sample to proceed to aetiological diagnosis, to align with previous work on ovine milk microbiology (Fthenakis 1994; Lafi *et al*. 1998; Vasileiou *et al*. 2018). However, a more liberal threshold of≥ 3 colonies was eventually applied, including the plates initially processed with the ≥10 colonies threshold.

10.3.2 Subclinical mastitis and aerobic plate count

The APC test involves plating the milk sample with agar into a petri dish and incubating for 72 hours. An alternative to the APC test is the BactoScan. The BactoScan is an automated instrument which does not require incubation like the plating method. Results have a rapid turnaround time of 24 hours. The test involves staining the bacterial DNA which is then detected and reported as a count. It is recommended by MilkTestNZ that sheep milk be tested with the conventional APC/SPC method.

10.3.2.1 On farm procedures

Selected ewes will be subject to a standardised clinical examination of the udder by visually inspecting it for signs of clinical mastitis (swelling, unevenness, discharging lesions) and palpating for swelling, pain, lumps, or heat. Any ewes with clinical mastitis will be excluded and replaced with another ewe.

Udders will be visually assessed for appearance and conformation and palpated for consistency and lumps (10.5.2.1). Teats will be measured (length and width at base) using callipers and graded for hyperkeratosis (10.5.2.2). Teat-end hyperkeratosis will be measured on a four-point scale developed for dairy ewes as described by Vouraki *et al*. (2018). During the analysis stage, the teat end scores will be categorised into three groups: Group 1 (no or mild hyperkeratosis) – ewes with both teat-ends scored <3; Group 2 (medium hyperkeratosis) – ewes with only one teat-end scored ≥3; Group 3 (severe hyperkeratosis) – ewes with both teat-ends scored ≥3.

Two strips from each half will be squirted onto a black plastic sheet to check for clinical mastitis. Then the teats will be cleaned according to EpiVets intramammary administration SOP (10.7) and two 3ml samples will be collected from each gland using aseptic technique,

re-cleaning the teats between samples. Specimen vials will be labelled with codes (see below) denoting the farm, ewe, gland and visit number. A second nonsterile 25ml sample will be collected from each gland for RMT, SCC and APC after the microbiology samples have been collected. Approximately 5ml from each gland will be used to perform a rapid mastitis test (RMT) while on farm, measured on a five-point scale (Fthenakis 1995). The remaining milk from the two glands will be combined into a single composite sample, gently mixed, and split into two subsamples for MilkTestNZ (SCC and APC testing, 30ml).

Ewe information and teat end and RMT data will be collected on Data Capture Form 3. The rapid mastitis test has been appraised for sheep. Using the 0, trace, 1, 2, 3 scale, an RMT score of 1 or above was deemed the optimal threshold for diagnosis of subclinical mastitis (Fthenakis 1995).

The sampling schedule is detailed below:

Sample Set	Month	Collection dates (Monday - Friday)	Testing dates (Tuesday - Saturday)
1	Aug-22	22/8/22 - 2/9/22	23/8/22 - 3/9/22
2	Nov-22	21/11/22 - 2/12/22	22/11/22 - 3/12/22
3	Feb-23	20/2/23 - 3/3/23	21/2/23 - 4/3/23

The following alphanumeric code system will be handwritten onto each microbiology vial to uniquely identify samples: [farm ID] [ewe ID] [gland] [visit]. E.g., farm A, ewe 254, left gland and second visit = A254L2.

The ewe identifications on the vials will be reconciled against the identifications recorded in Data Capture Form 3 to ensure alignment. Any discrepancies will be checked before the ewes are released. If discrepancies cannot be reconciled, the observations and samples will be discarded (and not used in the trial). If spare ewes are available (brought in as part of the protocol), they will be used.

All samples will be transported to the clinic at 2-7°C. The microbiology samples will be frozen before being shipped periodically to Massey University. Samples for MilkTestNZ will be transported in ice-packed containers on the same day as collection on farm to the laboratories, arriving within 48 hours of collection. MilkTestNZ will provide specific specimen vials with labels for this. The milk sample handling is summarised in 10.5.2.5.

10.3.2.2 Laboratory procedures

MilkTestNZ will perform a somatic cell count and an APC test at the ewe level (by combining gland samples into a composite sample) per their standard protocols.

Microbiology will be restricted to bacterial causes of mastitis. Contagious agalactia (caused by mycoplasma) will not be covered in this study. However, milk samples will be frozen and retained, should there be interest in mycoplasma. The same microbiology approach will be used as in 10.3.1.2 (clinical mastitis).

10.4 The dairy sheep mastitis app

All study farmers used a custom-built smartphone application to capture clinical mastitis case information. It was built with Jotform and populated an online database with study information. The app was also a repository of resources to help farms, including study guidelines and how-to videos. The instructions to install the app are:

How to install the mastitis app

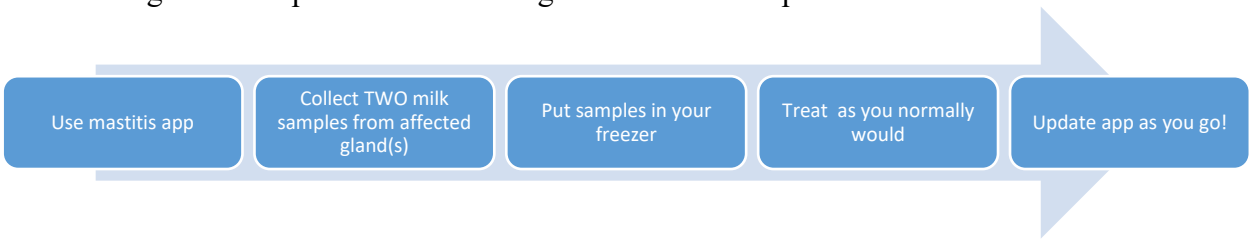
- Read this email from your smartphone
- On your smartphone, tap this link to open it on your phone: https://www.jotform.com/app/221517759848874
- Make sure you open the webpage in a web browser, as opposed to viewing it from within your email app (tap the Safari or Chrome icon for example in your mail app – whichever browser you use)
- On your phone, tap the box with the arrow pointing upwards and tap “Add to home screen” (there may be a different way of getting there for Android phones)
- Tap “add” (rename the app if you want to).
- Go to your phone’s home screen, open the app, and go!

10.5 Farmer and study operator guides

10.5.1 Clinical mastitis study protocol for farmers

This protocol covers everything you need to do when you find a case of clinical mastitis. All staff who might be detecting clinical mastitis will be trained before lambing starts in 2022 to ensure a consistent method across all farms.

Farm managers are responsible for training new staff on this protocol.



What is clinical mastitis

Clinical mastitis is:
A change in the appearance of **milk** (e.g., clots, blood, watery milk, red milk), **AND/OR**
Signs of inflammation in the **gland** (swelling, uneven udder, pain, lumps, discharging sores)

Clinical mastitis is NOT:

- Rapid mastitis test (RMT) positive ewes in the absence of the clinical signs above.
- Diseases limited to the teat or udder skin like scabby mouth, cuts, etc.

What to do when you find it

- Use your mastitis pack (see over)
- If you are treating the ewe, do not treat her until AFTER you have collected the milk samples

Procedure

1. Use the mastitis app (or other method as agreed)
2. Take a **digital photo** of the ewe from behind, capturing the whole udder, and upload it to the app (or **save** the photo with the ewe's identification number as the file name)
3. Collect and label **two** 10ml milk samples in separate containers **using the technique in the app/video/below**
4. Put the samples in your **freezer**
5. **Treat** as usual (if applicable - this study does not dictate how you treat ewes)
6. **Update mastitis app/form** as you go

When to look for it

At lambing/first milking
At the first time they are handled after lambing, check every ewe for clinical mastitis by:

- Stripping both glands AND
- Visually inspecting the udder for swelling, unevenness, lumps, sores

Any other time
We will not direct you on when to check for clinical mastitis. Please follow your usual processes or the advice of your vet.

How to collect milk samples
Watch the 3-minute video on the app or at https://youtu.be/yqrFIzDTKVY

Before you start
1. Wear disposable gloves and change gloves between animals (and between glands if affected in both glands)
2. Place **two** specimen jars per case within reach and loosen the lid but keep the lid on
3. Fill out the labels on the clipboard and then stick to the specimen jars (dry jars with paper towels if wet)
4. Write the ewe's ID on the lid in case the stickers come off during freezing

Only remove the lid immediately before collecting the sample (don't remove the lids off all the pottles at the start of the job).

Collecting the sample
1. Firmly scrub lower half of the teat with teat wipes and continue until the wipe comes away clean, using at least one wipe per teat
2. Remove the lid from the specimen jar and place it so the lid underside (the side facing the milk sample) cannot be contaminated
3. Hold the open jar at a 45-degree angle
4. Strip 10mL of milk into the jar
5. Store sample in freezer

Please do not
Let dirt/moisture drop into the pottle.
Let the ewe's teat touch the pottle.
Touch the inside of the pottle or lid.
Collect a sample if water is dripping from the udder/teat.

If any of these happen, please start again from the beginning.

Your mastitis pack

- Mastitis forms (if you are using them)
- Folder
- Specimen jars
- Paper towels (for drying wet specimen jars before placing stickers)
- Labels for the specimen jars
- Clipboard for in the shed
- Disposable gloves
- Marker pens
- Teat wipes
- Digital thermometer

Order more equipment through the app or by contacting sarah@epivets.co.nz.

If you have any questions, please use the "handy resources" in the app or contact Greg Chambers at greg@epivets.co.nz or 027 416 7865

10.5.2 Guides for study personnel

10.5.2.1 Udder and teat palpation guide

Scoring Guide

Udder Palpation

1	Diffuse soft consistency
2	Diffuse firm consistency
3	Soft consistency with small nodule(s) (lumps) - < 2cm in size
4	Soft consistency with large nodule(s) (lumps) - > 2cm in size
5	Firm consistency with small nodule(s) (lumps) - < 2cm in size
6	Firm consistency with large nodule(s) (lumps) - > 2cm in size
7	Diffuse hard consistency

Teat Palpation

- Can have more than one option circled

1	Soft consistency
2	Thickened teat orifice (end of teat)
3	Hard consistency
4	Palpation of a dense, vertical cord in the centre of the teat (feels like a straw)
5	Teat orifice obstruction (blind teat) *

10.5.2.2 Teat end hyperkeratosis scoring guide

The scale (1–4) developed for dairy ewes per Vouraki *et al.* (2018) will be used (see figure below).

Score	Description
1	No keratin ring around teat orifice
2	A smooth or slightly rough ring around the orifice and no keratin fronds
3	A raised roughened ring with isolated fronds of old keratin extending 1-3mm from orifice
4	A raised ring with rough fronds of old keratin extending >4mm from orifice

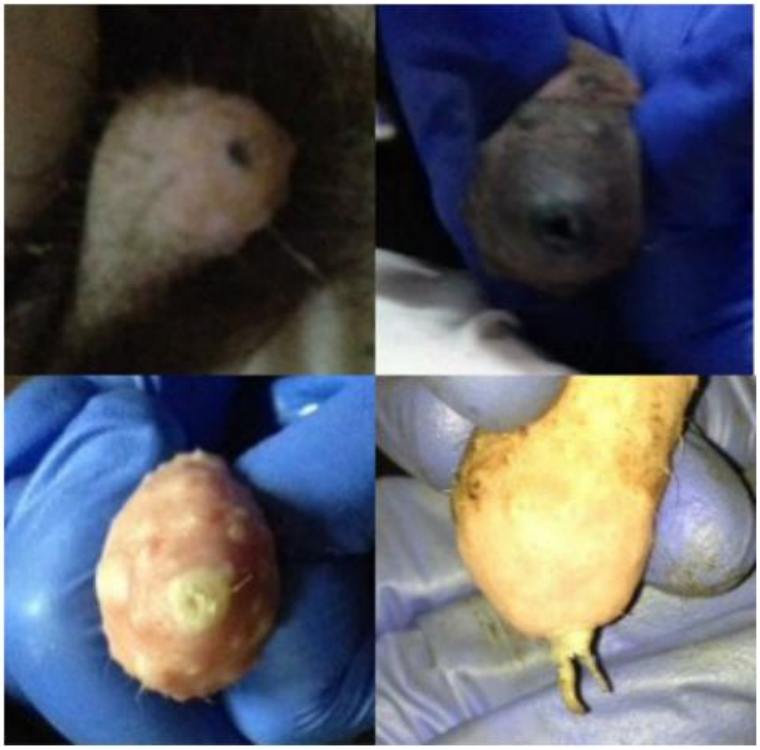


Fig. 1. Teat-end hyperkeratosis scores of ewe teat-ends: score 1 (top left) – no keratin ring around the teat orifice; score 2 (top right) – a smooth or slightly rough ring around the orifice and no keratin fronds; score 3 (bottom left) – a raised roughened ring with isolated fronds of old keratin extending 1–3 mm from the orifice; and score 4 (bottom right) – a raised ring with rough fronds of old keratin extending >4 mm from the orifice.

Image reproduced from Vouraki *et al.* (2018).

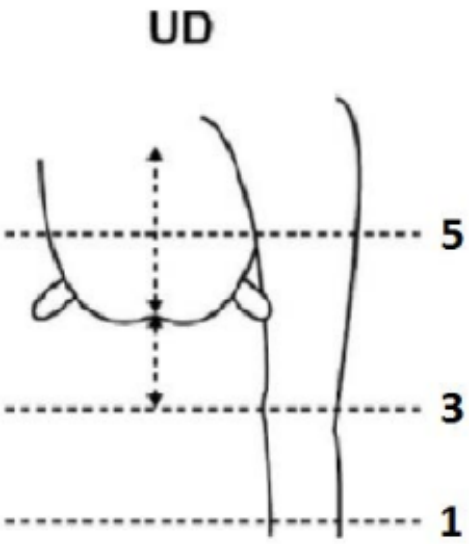
Following teat end assessment, the ewes will be assigned into three groups: Group 1 (no or mild hyperkeratosis) – ewes with both teat-ends scored <3; Group 2 (medium hyperkeratosis)

– ewes with only one teat-end scored ≥3; Group 3 (severe hyperkeratosis) – ewes with both teat-ends scored ≥3.

10.5.2.3 Standing udder measurement guide

Scoring Guide

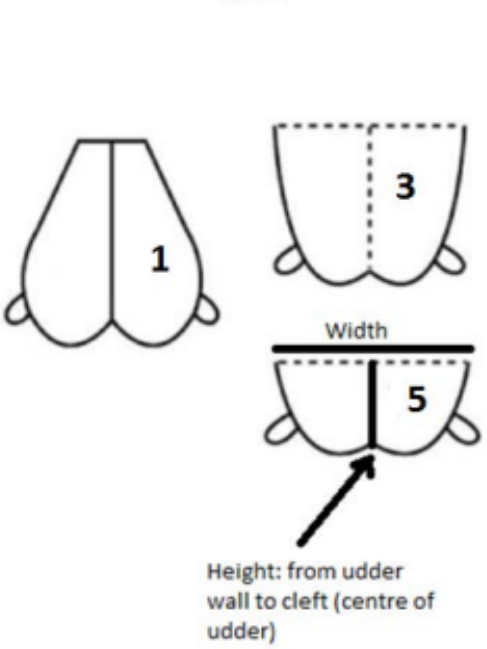
Udder Depth (UD)



UD = The distance between the udder cleft and the abdominal wall, taking as a reference the line joining the hocks

E.g. A score of 3 = hock level

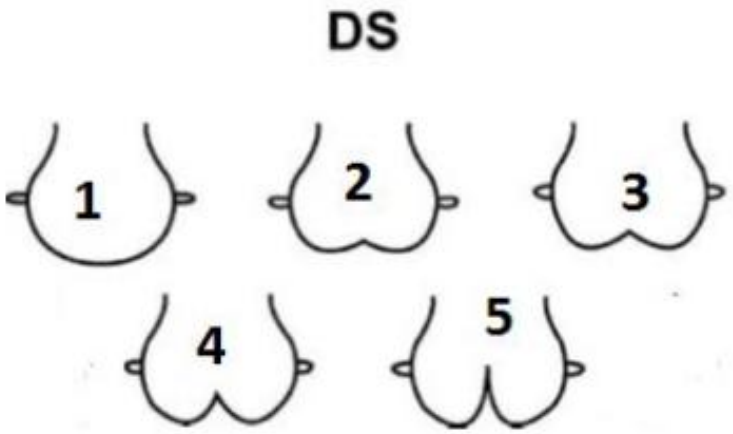
Suspension (SU)



SU =Degree of suspension of the udder. The ratio between the udder attachment width and height.

For a score of 5 the attachment width is much larger than the udder depth, for a score of 3 the udder is apparently "square" and for a score of 1 the udder width is much narrower than the udder depth.

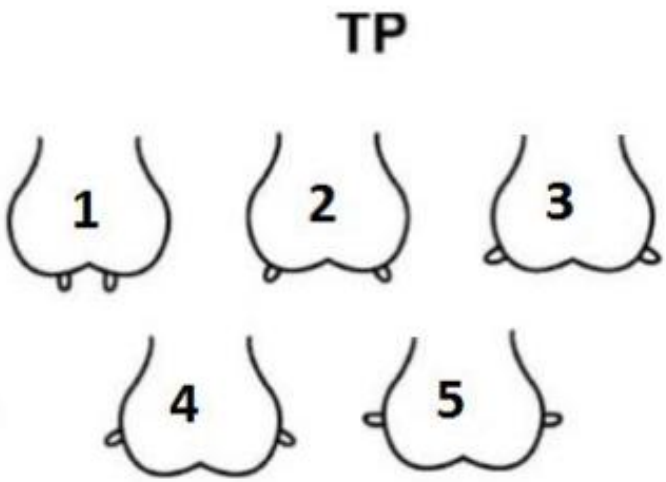
Degree of Separation (DS)



DS = Degree of separation of the 2 halves.

For a score of 1 there is no separation vs. for a score of 5 the udder is clearly divided into 2 halves

Teat Placement (TP)



TP = Teat Placement

The external height of the cistern i.e. the distance between the teats and the lowest part of the udder

10.5.2.4 RMT interpretation guide

Using proprietary RMT paddles, an equal volume of proprietary RMT detergent and milk will be mixed in each paddle for each gland and swirled for 15 seconds. The solution will then be graded according to Barnum and Newbould (1961) as follows:

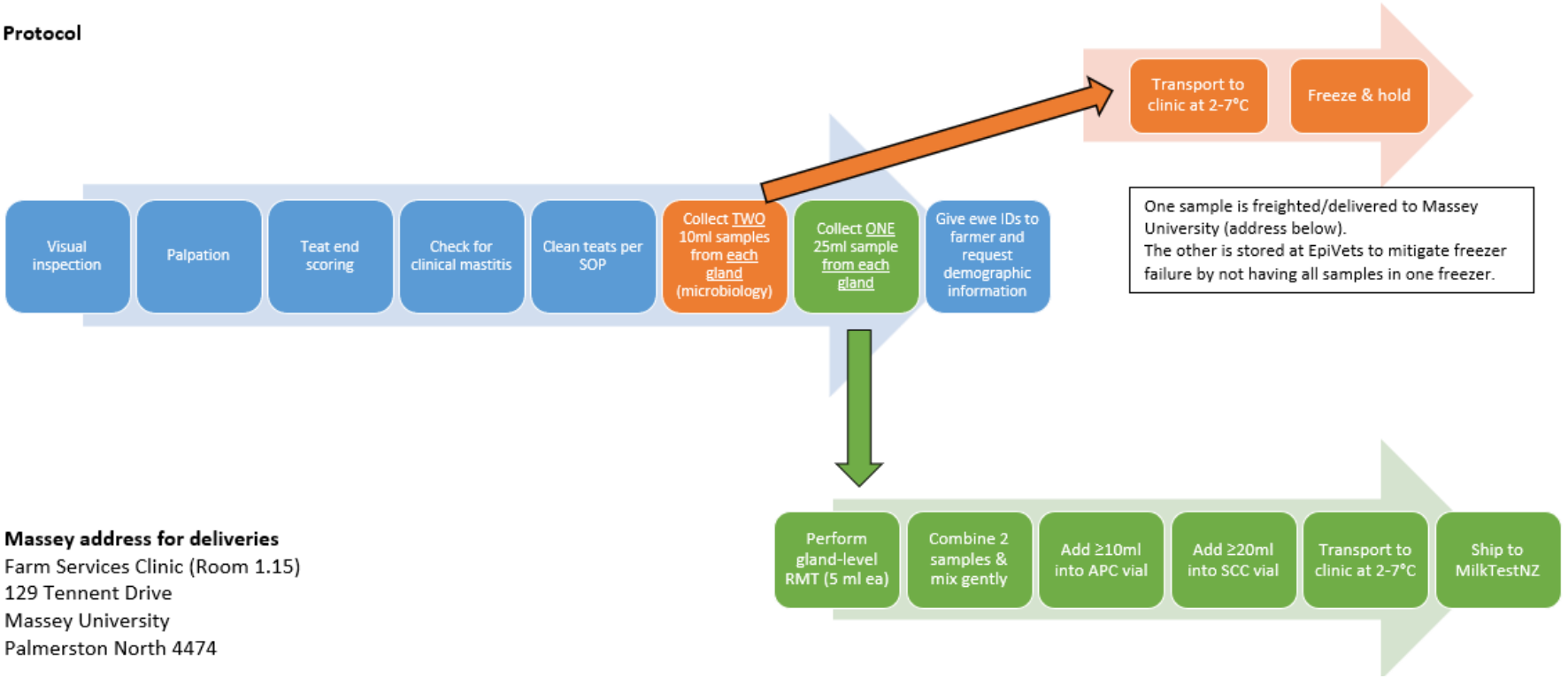
Score	**Description**
0 (negative)	No change in consistency
T (trace)	No visible change in consistency, but when paddle is tipped a slime is momentarily seen on the bottom
1	A gel or thick slime forms, but when paddle is swirled the solution does not move into the centre
2	A thick lumpy gel forms, which, when swirled, quickly moves toward the centre
3	A distinct gel forms which tends to adhere to the bottom of the paddle, and during swirling a distinct central peak forms

Background

Each study farm will be visited by technicians three times during the 2022/2023 season. We will examine and sample 30 randomly selected ewes, typically during milking (some farmers will draft them out before milking). Using a random number generator, the position of the first ewe to be examined will be selected, and then every n/30th ewe will be examined (n=milking flock size). If a ewe is excluded (e.g., clinical mastitis), the next ewe will be sampled. Then the n/30th ewe after the ewe that was excluded will be sampled.

The ewes' udders will be visually assessed and palpated, the teat ends scored, and then milk samples collected for RMT, SCC, APC, and culture.

Protocol



Massey address for deliveries

Farm Services Clinic (Room 1.15)
129 Tennent Drive
Massey University
Palmerston North 4474

10.6 Data Capture Forms

10.6.1 Data Capture Form 1

Clinical mastitis case information

Please enter as much information as you can so we can give you useful research findings. If you do not know the answer, please do your best to find it. If you still cannot answer any questions, enter "unknown". Do not enter guesses.

Basic information

Farm name:
Your name:
Ewe ID (tag or EID number):
Ewe age (years):
Date of mastitis detection:
Affected gland(s) (left, right, both):
Number of lambs at scanning*:
Number of lambs born (1, 2, 3, 4+, unknown):
Lambing date (if known):
First milking date (if known):
Did it rain on two or more days in the week before mastitis was detected? (Y/N):
Where did the ewe lamb? (Indoors, outdoors, unknown):

* 0, 1/single, 2/twins, 3, 4 or more, "multiple" (2 or more), "multiple" (3 or more but not twins), unknown

Milk

Clots (Y/N)	
Colour (white/yellow/pink/red/watery)	
Did you notice a drop milk yield in the 1-2 days before detection? (Y/N)	

Gland – *please take a digital photo of the udder from behind and email it to greg@epivets.co.nz with your name, the farm name, the date mastitis was detected, and the ewe's ID number*

Painful? (Sensitive to touch, difficulty walking) (Y/N)	
Swollen? (Y/N)	
Uneven gland sizes? (Y/N)	
Lumps in the affected gland? (Y/N)	
Gangrene? (Cold or peeling skin, (Y/N)	

Ewe

Rectal temperature (°C)	
Depressed (slow, not eating, unable to stand)? (Y/N)	

Treatments given *(if applicable)*

Date	Product	Dose (ml)	Route*	Site (neck/rump/other)

* Intramuscular (IM), Subcutaneous (under the skin – SC), Other (e.g. Intramammary)

Outcomes *(tick one)*

Still milking: full recovery without lasting effects	
Still milking: recovered but with lasting effects (e.g., weak gland)	
Dried off: full recovery without lasting effects	
Dried off: recovered but with lasting effects (e.g., weak gland)	
Culled: due to lasting mastitis problems	
Culled: due to non-mastitis problems	
Died: due to mastitis	
Died: due to non-mastitis problems	
Other outcome (describe below)	

Lasting effects *(if applicable)*

Other outcome *(if applicable)*

Dates of drying off/culling/death

Date dried off (if applicable)	
Date culled (if applicable)	
Date of death (if applicable)	

10.6.2 Data capture form 3

Farm			
Date			
Ewe ID			
		LEFT	**RIGHT**
Inflammation	**Udder**	Yes / No	Yes / No
	Teat	Yes / No	Yes / No
Lesion	**Udder**	Yes / No scab, scar, nodule	Yes / No scab, scar, nodule
	Teat	Yes / No scab, scar, nodule	Yes / No scab, scar, nodule
Palpation	**Udder**	1 / 2 / 3 / 4 / 5 If 3+, n nodules: 1 / 2 / 3+	1 / 2 / 3 / 4 / 5 If 3+, n nodules: 1 / 2 / 3+
	Teat	1 / 2 / 3 / 4 / 5 If 3+, n nodules: 1 / 2 / 3+	1 / 2 / 3 / 4 / 5 If 3+, n nodules: 1 / 2 / 3+
	Any other comments		
Measurements	**Teat length (mm)**		
	Teat width (mm)		
Hyperkeratosis		1 / 2 / 3 / 4	1 / 2 / 3 / 4
Udder depth	**Score**	1 / 2 / 3 / 4 / 5	
Suspension	**Score**	1 / 2 / 3 / 4 / 5	
Degree of separation	**Score**	1 / 2 / 3 / 4 / 5	
Teat placement	**Score**	1 / 2 / 3 / 4 / 5	
Symmetrical	**Score**	Yes / No	
	Detail if no		
BCS	**Score**		
RMT		0 / T / 1 / 2 / 3 / 4	0 / T / 1 / 2 / 3 / 4
Notes			

10.6.3 Data Capture Form 6

Farm name: **Date:**

	Ewe ID	Age (yrs)	Breed	Lambing date	First milking date	No. lambs born
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						

10.7 Teat cleaning and milk sampling SOP

10.7.1 Definitions

- Principal Investigator: The EpiVets Veterinarian responsible for heading the Research team.
- Technician: The Technician employed by or contracted to EpiVets.
- Veterinarian: A Veterinarian employed by EpiVets responsible for training Technicians. It is possible that more than one Veterinarian is involved with a particular Technician's training.
- Drug: In this SOP the reference to the word "drug" implies either an antibiotic or a teat sealant.

10.7.2 Procedure

- All equipment will be laid out in a space that is protected from contamination by water droplets, urine, and other contaminants
- The trained Technician will wash and disinfect their hands and apply clean latex rubber gloves to both hands
- The ewe's ID and the gland will be handwritten on each vial with a permanent marker pen prior to collecting the sample. Adhesive labels with a code will be applied to each vial after collecting samples at the clinic. The code is: [farm ID] [ewe ID] [gland] [visit]. E.g., farm A, ewe 254, left gland and second visit = A254L2.
- Lids will remain on specimen jars until immediately before sampling
- The teat will be disinfected by rubbing the teat end with a premedicated wipe of the same kind used for administering intramammary products to cows, focusing on the teat end.

- The teat end will be scrubbed until free of gross/visible contaminants. The teat end will then be scrubbed again with a fresh wipe, continuing until the wipe comes away clean, using as many wipes as needed.
- Remove the lid from the jar and place it so the lid underside (the side facing the milk sample) cannot be contaminated
- Hold the open jar at a 45-degree angle
- Strip 10mL of milk into the jar
- The teat will be re-cleaned per steps 6 and 7 above between samples, when collecting duplicate samples.
- Change gloves between ewes, or if contaminated between glands of the same ewe.
- Samples will be stored at 2-7°C during transport to the clinic, whereupon they will be frozen at -20°C.
- If any of the following occur, the sample must be discarded, the teat re-cleaned, and new samples collected:
- Dirt or moisture drop into the jar.
- The ewe's teat touched the jar.
- The technician touches the inside of the jar or lid.
- Water drips from the udder/teat.

10.8 References

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