PREVALENCE AND PRODUCTION IMPACTS OF SUBCLINICAL MASTITIS IN EXTENSIVELY MANAGED EWES

by

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ABSTRACT

Mastitis results from a bacterial infection of the mammary gland and is a devastating disease to all sheep producers from both an economic and animal welfare perspective. Clinically infected ewes display visually apparent symptoms, however, subclinically infected ewes do not although this form is more common. Since milk is a direct commodity of dairy animals, much of the past research has been conducted here and the production and economic impacts of subclinical mastitis are less clear in non-dairy (e.g., meat- and wool-type) ewes. The objectives of the first study were to identify bacteria species present in milk collected from clinically healthy ewes and evaluate somatic cell count (SCC) thresholds relating to intramammary infection. Milk samples were collected from two research flocks in the Western U.S. (Montana State University = MSU; U.S. Sheep Experiment Station = USSES). Bacteria were identified by both culturing and identification via mass spectrometry and polymerase chain reaction methods. Overall, 60 bacteria species were identified using mass spectrometry and the most common belonged to the *Bacillus* and *Staphylococcus* genera. The ideal SCC thresholds to predict intramammary infection ranged between 240 x 10^3 to 1370 x 10^3 cells/mL, depending on the flock and time of collection. In the second study, milk samples were collected and udder and teat morphometric traits were observed to predict ewe productivity via dam 120 day adjusted litter weaning weight (LW120). Udder and teat characteristics were assessed on a linear scale at each sampling and included teat length, udder symmetry, and presence of supernumerary teats, to name a few. The effect of $\log_{10}$-transformed SCC (LSCC) on ewe productivity was dependent on lactation stage and production year, but when significant, indicated a 9.2-14.7 kg reduction in LW120 associated with a 1-unit increase in LSCC. Factors which influenced LSCC included parity, production year, and presence of supernumerary teats in USSES ewes and, for MSU ewes, included teat length, external teat damage, udder symmetry, and presence of supernumerary teats. The results indicate subclinical mastitis is common and additional studies investigating techniques to mitigate its severity and prevalence in meat- and wool-type ewes are warranted.

CHAPTER ONE

INTRODUCTION

Mastitis can be defined as an inflammation of the mammary gland that is often caused by bacterial infection (Kahn et al., 2010). The term mastitis is originally derived from the Greek prefix masto-, meaning breast or mammary gland, and the Latin suffix -itis, meaning a specified part is inflamed. Since mastitis is an inflammation of the mammary tissue, the disease is present and important in all mammalian species. However, since milk is a direct commodity of dairy animals, the majority of mastitis research has been conducted in these species (Bergonier and Berthelot, 2003; Bergonier et al., 2003; Contreras et al., 2007).

Mastitis is most often categorized into either clinical or subclinical states (Kahn et al., 2010). Clinical mastitis can be easily diagnosed as infected animals typically express milk that is abnormal in color, consistency, and may include the presence of fibrin clots. Furthermore, a clinically infected udder usually becomes swollen, firm, and feverish. Additionally, clinically infected ewes may behave abnormally, such as becoming depressed and restless (Fthenakis and Jones, 1990a). Clinical mastitis is referred to as mild or moderate if symptoms remain local. If the inflammatory response initiates systemic changes, such as an elevated body temperature, reduced feed intake, or shock, this state of mastitis is referred to as severe clinical (Kahn et al., 2010).

Unlike clinical mastitis, subclinical mastitis presents no observable symptoms in the milk, the udder, or the behavior of the infected animal (Kahn et al., 2010). However,

past researchers have reported higher incidences of ewe culling after experiencing subclinical mastitis and reduced lamb performance in terms of survival and growth. Intramammary infection (IMI) status, and thus subclinical mastitis, can only be diagnosed through bacterial screening of milk samples. However, the somatic cell count (SCC) of a milk sample is commonly used to infer IMI in dairy animals (Raynal-Ljutovac et al., 2007; Fragkou et al., 2014) and will be a major focus of this thesis. The objectives of this thesis were to: a) identify bacterial species present in milk and evaluate SCC thresholds relating to IMI in clinically healthy ewes managed in extensive production systems and b) estimate the effect of somatic cell count (SCC) on ewe productivity and relationships among udder and teat morphometric traits and SCC in 2 Western U.S. research flocks.

CHAPTER TWO

LITERATURE REVIEW

Introduction

Mastitis, both in clinical and subclinical states, is often the result of bacterial infection (Kahn et al., 2010) and contributes to financial losses due to ewe culling (USDA APHIS 2011), veterinary treatments (Timms, 2007), and reduced milk yield (Torres-Hernandez and Hohenboken, 1979; McCarthy et al., 1988; Fthenakis and Jones, 1990b). Earlier work estimated that mastitis costs the U.S. sheep industry between $20-25 million annually (Smith, 1989; Ahmad et al., 1992a), however, a thorough analysis of the economic impact of subclinical mastitis on the U.S. sheep industry has not been conducted. Still, past research has linked the disease to reduced lamb performance. For example, lambs reared by infected ewes have reduced survival rates (Holmøy et al., 2014) and are slower growing (Gross et al., 1978; Fthenakis and Jones, 1990b; Ahmad et al., 1992a). Bacterial pathogens have been commonly isolated from ewe milk from clinically healthy animals, although their identities and frequencies are widely variable across literature. In dairying systems, somatic cell count (SCC) thresholds are often used to infer subclinical mastitis, since infected animals display no visually apparent symptoms. However, in non-dairy ewes, there has not been a clear threshold to infer subclinical mastitis in past literature, and the only true diagnostic method is to isolate bacteria present in milk. Past researchers have developed linear scoring systems to

evaluate and select for animals with desirable udder characteristics, although genetic and phenotypic correlations vary widely across traits (de la Fuente et al., 1996; Serrano et al., 2002; Casu et al., 2006). Additional management practices, such as antibiotic therapy or post-weaning fasts, have been evaluated to reduce the prevalence and severity of subclinical mastitis (McCarthy et al., 1988; Hueston et al., 1989; Fthenakis et al., 2012). The following sections will review past mastitis literature with particular emphasis on the most common pathogens isolated from clinical and subclinical cases in sheep, milk SCC thresholds corresponding to subclinical infection, risk factors that may predispose ewes to infection, economic implications on ewe and lamb productivity, and husbandry practices to mitigate its effects.

Etiological Agents

Mastitis is most often caused by a bacterial infection, but there is also evidence that neoplasms, lentiviruses, and allergens can be causative agents (Pekelder et al., 1994; Menzies and Ramanoon, 2001; Kahn et al., 2010). Nevertheless, the focus of this thesis will be on the etiology and production impacts of bacterial mastitis. The following sections titled “Gram-Positive Bacteria” and “Gram-Negative Bacteria” are generally organized from most to least commonly isolated genera reported in the literature.

Gram-Positive Bacteria

Gram-positive bacteria have cell walls composed of peptidoglycan that are thicker than Gram-negative bacteria (Singleton and Sainsbury, 2001). Peptidoglycan provides strength to the cell wall, thus giving the cell its shape (Singleton and Sainsbury, 2001).

Gram-positive bacteria tend to be susceptible to penicillin and detergents (Tortora et al., 2013). Several species of Gram-positive bacteria that have been isolated from clinical and subclinical cases of ovine mastitis are discussed below.

Staphylococcus spp. *Staphylococcus* spp. are facultative anaerobic organisms that belong to the phylum Firmicutes (Singleton and Sainsbury, 2001). These bacteria are approximately 1 µm in diameter, group together to resemble grape clusters, and are common on the skin and along the respiratory, urogenital, and digestive tracts (Quinn et al., 2011). *Staphylococcus* spp. are common etiological agents that cause mastitis, especially in cattle (Quinn et al., 2011). Most infections are subclinical, though some may be acute or chronic (Quinn et al., 2011). Field studies evaluating the efficacy of different vaccines against intramammary infections and mastitis caused by *S. aureus* have had varying results in dairy cattle (Landin et al., 2015). In ewes, vaccinations have reduced the incidence of clinical and gangrenous mastitis, albeit there were less evident impacts on subclinical mastitis (Watson, 1988; Amorena et al., 1994). *Staphylococcus* spp. are further classified as coagulase-negative or -positive, depending on whether the bacterium produces coagulase (Singleton and Sainsbury, 2001), which protects the cell from oxidative damage of reactive oxygen species.

Coagulase-Negative Staphylococci Of the mastitis causing *Staphylococcus* spp., many are coagulase-negative staphylococci (CoNS). Globally, *S. epidermidis* is the most common CoNS that causes subclinical mastitis in ewes and is associated with the greatly

elevated SCC in both does and ewes (Bergonier et al., 2003). Other common CoNS include *S. xylosus, S. chromogenes,* and *S. simulans*.

Several studies have isolated CoNS in both clinically and subclinically infected dairy ewes. In subclinically infected Churra ewes across 8 Spanish flocks, the most common CoNS isolates included *S. epidermidis* (53.2%), *S. xylosus* (7.0%), and *S. chromogenes* (4.1%; Ariznabarreta et al., 2002). Similarly, Spanu et al. (2011) collected milk from East Friesian and Lacaune influenced ewes at parturition and 2 to 3 wk later and, within culture-positive samples, identified CoNS in 45.0 and 47.5%, respectively. Furthermore, *S. xylosus* (11.9 and 8.5%), *S. chromogenes* (9.2 and 3.4%), *S. epidermidis* (9.2 and 3.4%), *S. auricularis* (3.7 and 11.9%), and *S. simulans* (3.7 and 3.4%) were most common at these two time points, respectively. In subclinically infected dairy ewes (Churra, Castellana, and Assaf) from18 Spanish flocks, CoNS species were identified in 62.5% of bacterial isolates (González-Rodríguez et al., 1995). De la Cruz et al. (1994) reported that, of subclinically infected Manchega ewes, CoNS were isolated in 78.6% and *S. epidermidis* was most common (66.8%). However, much lower frequencies of CoNS (17.9%) within culture-positive Awassi samples were reported by Al-Majali and Jawabreh (2003). In a larger study of clinically and subclinically infected Awassi ewes across 20 flocks in Jordan, CoNS species were cultured in 13.1 and 25.6% , respectively, of bacteriologically positive milk samples (Lafi et al., 1998).

In addition to being isolated in mastitis cases in dairy sheep, CoNS have also been isolated in meat-type ewes rearing their own lambs. In Scottish crossbred ewes with subclinical mastitis, CoNS species were isolated in 69% of bacteriologically positive milk

samples, and *S. equorum* (41.3%), *S. xylosus* (15.2%), and *S. simulans* (13.0%) were the most common (Hariharan et al., 2004). A similar prevalence was reported by Clements et al. (2003), who identified CoNS species in 71.4% of culture-positive milk samples collected from subclinically infected Scottish mule ewes, including *S. cohnii* (19.0%), *S. sciuri* (14.3%), *S. epidermidis* (9.5%), and *S. warneri* (9.5%). However, Mørk et al. (2007) reported a much lower incidence of CoNS (2.9%) in clinically infected crossbred ewes in Norway. Still, Arsenault et al. (2008) isolated CoNS from 13.6 and 28.9% of culture-positive clinical and subclinical cases, respectively. Therefore, CoNS are a common causative pathogen of mastitis in many flocks and breeds.

Coagulase-Positive Staphylococci Coagulase-positive species of staphylococci that are causative agents of ovine mastitis include *S. aureus*, *S. hyicus*, *S. intermedius*, and *S. schleiferi* (Gelasakis et al., 2015). Of these, it has been estimated that *S. aureus* is responsible for approximately 40% of mastitis cases in nursing ewes and 80% of cases in lactating dairy sheep (Lafi et al., 1998; Mørk et al., 2007). González-Rodríguez et al. (1995) identified coagulase-positive staphylococci in 11.4% of isolates from subclinically infected dairy ewes. In additional studies previously reported, *S. aureus* was isolated from culture-positive samples of subclinically infected dairy ewes at a frequency of 9.8% (Lafi et al., 1998) and 33.9% (Al Majali and Jawabreh, 2003). Additionally, Lafi et al. (1998) and Marogna et al. (2010) identified *S. aureus* in 31.8 and 13.5% of clinically infected dairy ewes, respectively. However, other studies have identified *S. aureus* in less than 5% of bacterial isolates in milk collected from subclinically infected dairy ewes (de la Cruz et al., 1994; Ariznabarreta et al., 2002; Spanu et al., 2011), suggesting variation in

the frequency of the bacterium between breeds and flocks. Interestingly, Saratsis et al. (1998) was able to identify *S. aureus* in non-lactating dairy ewes across 10 Greek flocks at a frequency of 19.1 and 8.6% 2 to 3 weeks before parturition and 3 weeks after milking ceased, respectively.

Coagulase-positive staphylococci have also been isolated in cases of mastitis in meat- or wool-type ewes. *Staphylococcus aureus* was isolated in 40% of culture-positive milk samples collected from 8 Australian flocks of various breeds (Watson et al., 1990). However, other researchers have isolated the bacterium at much lower frequencies in cases of subclinical mastitis, including 8.3% (Watkins et al., 1991) and 2.3% (Hariharan et al., 2004). Still, Arsenault et al. (2008) identified the species in 36.4 and 30.6% of positive cultures from clinically and subclinically infected ewes, respectively. *Staphylococcus aureus* has been isolated in cases of clinical mastitis, including in 44.8% and 76.4% of samples from Norwegian crossbred (Mørk et al., 2007) and Texel ewes (Koop et al., 2010), respectively. In conclusion, although coagulase-positive staphylococci have been more commonly isolated in dairy ewes than in non-dairy ewes, they are still prevalent in ovine IMI.

Streptococcus spp. *Streptococcus* spp. are facultative anaerobic organisms that belong to the Firmicutes family (Singleton and Sainsbury, 2001). These bacteria are coccoid, approximately 1.0 µm in diameter, and form chain links (Quinn et al., 2011). Many species inhabit the respiratory and urogenital tracts, but only survive for short durations off the host (Quinn et al., 2011).

Of the previously described studies, *Streptococcus* spp. were identified in 25.0% (Al-Majali and Jawabreh, 2003), 16.2% (González-Rodríguez et al., 1995), and 14.6% (Lafi et al., 1998) of subclinically infected dairy ewes. However, others have reported lower frequencies of *Streptococcus* spp. in subclinically infected ewes, including 3.1% (Ariznabarreta et al., 2002), 1.5% (de la Cruz et al., 1994), and between 1.6 to 5.5% (Spanu et al., 2011). In clinically infected dairy ewes, frequencies of *Streptococcus* spp. were 9.3 and 25.6% (Lafi et al., 1998; Marogna et al., 2010).

Streptococcus spp. have also been isolated from meat-type ewes with subclinical mastitis. While several of the previously described studies investigating subclinical ewes identified the species at high frequencies (15.9% in Hariharan et al., 2004; 41.7% in Watkins et al., 1991), others did not (9.5% in Clements et al., 2003; 5.6% in Arsenault et al., 2008). In Europe, Watson et al. (1990) isolated the bacteria in 12.9% and Kern et al. (2013) in 23% of positive cultures. However, Blagitz et al. (2014) isolated *Streptococcus* spp. in few culture-positive samples (6.8%) across 17 Brazilian Santa Ines flocks. In non-dairy ewes with clinical mastitis, the frequency of the genus is low (5.4% in Mørk et al., 2007; 3.4% in Koop et al., 2010). Therefore, *Streptococcus* spp. have been reported to be common causative agents of both subclinically and clinically infected ewes across many flocks.

Bacillus spp. *Bacillus* spp. are rod shaped firmicutes, range in size from 0.5 to 2.5 x 1.2 to 10 µm, and form endospores. They are either strictly aerobic or facultative anaerobic species and found in soil (Singleton and Sainsbury, 2001). In dairy ewes with subclinical mastitis, *Bacillus* spp. were isolated from 5.4% (Al-Majali and Jawabreh,

2003) and 5.1-6.4% (Spanu et al., 2011) of bacteriologically positive milk. Similar results have been found in meat-type ewes with subclinical mastitis in England (4.2%; Watkins et al., 1991) and Canada (4.5%; Arsenault et al., 2008). However, Arsenault et al. (2008) also reported a *Bacillus* spp. frequency of 20.8% in clinical mastitis cases. Still, Watson et al. (1990) isolated the bacterium in 5.9% of bacteriologically positive samples. Hence, *Bacillus* spp. are causative agents of mastitis in low to moderate frequencies.

Trueperella spp. *Trueperella pyogenes* is a pleomorphic and facultative anaerobic microbe that is part of the Actinobacteria phylum (Singleton and Sainsbury, 2001) and commonly found on mucus membranes (Quinn et al., 2011). The species was once classified as *Corynebacterium pyogenes, Actinomyces pyogenes,* and *Arcanobacterium pyogenes*, but was later reclassified. Saratsis et al. (1998) isolated *T. pyogenes* in 27.2 and 23.5% of non-lactating dairy ewes 2-3 weeks before parturition and 3 weeks after lactation ended, respectively. Al-Majali and Jawabreh (2003) isolated the bacterium in 8.9% in dairy ewes. However, frequencies less than 5% have been reported in both clinically and subclinically infected dairy ewes (Lafi et al., 1998). In clinically infected meat-type ewes, Mørk et al. (2007) isolated the species in less than 1% of cases. Therefore, *T. pyogenes* has been a common causative agent of both clinical and subclinical mastitis in some flocks but may be less common in others.

Less Common Gram-Positive Species *Aerococcus* spp. belong to the family Streptococcaceae. They are non-motile cocci that are most commonly grouped together

as pairs or tetrads (Singleton and Sainsbury, 2001). Hariharan et al. (2004) isolated the genus in 2.1% of subclinically infected non-dairy ewes with single pathogen isolates.

Clostridium spp. are classified as firmicutes, range in size from 0.3 to 1.9 x 2 to 10 µm, and are obligate anaerobic organisms (Singleton and Sainsbury, 2001). Bacteria are endospore forming, rod shaped, coccoid, or filamentous. Species can be found in soil and in intestines, and some may be opportunistic pathogens (Singleton and Sainsbury, 2001). Mørk et al. (2007) isolated *C. perfringens* in 1.5% of clinically infected meat-type ewes.

Enterococcus spp. are facultative anaerobic bacteria that belong to the Firmicutes phylum. They are generally 0.6-2.0 x 10-20 µm in size, coccoid in shape, and inhabit the intestines (Singleton and Sainsbury, 2001). *Enterococcus* spp. have been isolated at frequencies less than 5% in clinically or subclinically infected dairy sheep (Ariznabarreta et al., 2002; Marogna et al., 2010) and non-dairy sheep (Clements et al., 2003; Hariharan et al., 2004; Arsenault et al., 2008).

Micrococcus spp. are aerobic bacteria belonging to the Actinobacteria phylum, coccoid in shape, group together in tetrads or clusters, range in size from 1 to 2 µm in diameter, and found on the skin (Singleton and Sainsbury, 2001). In dairy ewes with subclinical mastitis, the genus has been isolated in less than 5% of cases (de la Cruz, 1994; González-Rodríguez et al., 1995; Saratsis et al., 1998; Ariznabarreta et al., 2002). In subclinically or clinically infected wool- or meat-type ewes, Watson et al. (1990) identified the genus in 11.8% of isolates, although Kern et al. (2013) identified the genus in only 1.7% of isolates.

Nocardia spp. are facultative anaerobic bacteria belonging to the Actinobacteria phylum (Singleton and Sainsbury, 2001). They are generally 0.5-1.2 µm in diameter and form branching filaments that fragment into cocci or rods (Quinn et al., 2011). In meat-type ewes, Arsenault et al. (2008) isolated the genus in 2.4% of subclinical cases and Watson et al. (1990) in fewer than 1% of clinical cases.

In conclusion, there are many Gram-positive bacteria that have been isolated in cases of ovine IMI in both dairy and non-dairy ewes, however, prevalence of species and genera are highly variable across flocks, years, and breeds. Still, commonly identified Gram-Positive bacteria include *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., and *Trueperella* spp. Less commonly isolated include *Aerococcus* spp., *Clostridium* spp., *Enterococcus* spp., *Micrococcus* spp., and *Nocardia* spp.

Gram-Negative Bacteria

Gram-negative bacteria have cell walls that are thinner and contain less peptidoglycan than Gram-positive bacteria, resulting in weaker cell walls (Singleton and Sainsbury, 2001). Furthermore, Gram-negative bacteria tend to be more resistant to antibiotics than Gram-positive bacteria (Tortora et al., 2013). Several species of Gram-negative bacteria that have been isolated from clinical and subclinical cases of ovine mastitis are discussed below.

Escherichia spp. *Escherichia* spp. are facultative anaerobic organisms which belong to the Proteobacteria phylum (Singleton and Sainsbury, 2001). *Escherichia coli* are generally straight rods with rounded ends, 0.5 x 1-4 µm, found singly or within pairs,

and commonly inhabit the intestinal tract (Quinn et al., 2011). Most *E. coli* strains are commensal but can be opportunistic outside the intestine (Quinn et al., 2011).

In subclinically or clinically infected dairy ewes, *E. coli* has been isolated in approximately 20% of ewes (Lafi et al., 1998; Al-Majali and Jawabreh, 2003). In meat-type ewes with subclinical mastitis, Watkins et al. (1990) isolated the species in 4.2% of cases. In clinically infected non-dairy ewes, Mørk et al. (2007) isolated *E. coli* in 7.5% of cases. Therefore, *E. coli* is a common causative pathogen of clinical and subclinical mastitis in both dairy and meat-type ewes.

Mannheimia spp. *Mannheimia* spp. are anaerobic microbes that belong to the phylum Proteobacteria (Singleton and Sainsbury, 2001). Cells are either coccobacillus or rod shaped and generally 0.3-1.0 x 1-2 µm in size (Singleton and Sainsbury, 2001; Quinn et al., 2011). *Mannheimia haemolytica* is a known pathogen to cause pneumonia in both cattle and sheep, mastitis in ewes, and septicemia in lambs (Singleton and Sainsbury, 2001). The species was once classified as *Pasteurella haemolytica* (Newsom and Cross, 1932), but was re-classified in 1999 after further investigations including phenotypic data, 16S r DNA sequencing, and DNA-DNA hybridization (Angen et al., 1997a, b & 1999a, b). *Mannheimia* spp. are considered the most common cause of clinical and subclinical mastitis in ewes rearing their lambs but is not common in dairy ewes (González-Rodríguez et al., 1995). Apart from *M. haemolytica*, *M. glucosida* is the only other species of *Mannheimia* known to cause mastitis in sheep (Arsenault et al., 2008; Omaleki et al., 2010).

Some researchers have isolated *M. haemolytica* at frequencies greater than 5% in subclinically infected meat-type ewes, including 22.9% (Watkins et al., 1991) and 9.5% (Clements et al., 2003). However, others have reported lower frequencies, including 2.4% (Arsenault et al., 2008) and 4.5% (Hariharan et al., 2004). Much greater frequencies have been reported in cases of clinical mastitis in meat-type ewes (40.9% in Arsenault et al., 2008; 39.1% in Koop et al., 2010). However, Mørk et al. (2007) isolated *M. haemolytica* in relatively few clinical cases in non-dairy ewes (1.8%). Hence, *M. haemolytica* can be a common causative pathogen of IMI in non-dairy ewes nursing their lambs, but variation in its frequency has been reported.

Less Common Gram-Negative Species *Acinetobacter* spp. are strictly aerobic microbes that belong to the phylum Proteobacteria (Singleton and Sainsbury, 2001). They are rod shaped, generally 1 x 1.5-2.5 µm, and group together in pairs (Singleton and Sainsbury, 2001). Watson et al. (1990) isolated the genus in samples collected from non-dairy ewes with an IMI in 4.7% of positive cultures.

Enterobacter spp. are facultative anaerobic organisms which belong to the phylum Proteobacteria (Singleton and Sainsbury, 2001). They are rod shaped, typically 0.6 x 1.2-3.0 µm in diameter, and found on plants, in soil, water, and sewage, and within the intestinal tract (Singleton and Sainsbury, 2001). In ewes with either clinical or subclinical mastitis, Kern et al. (2013) isolated the genus in 5.7% of samples across flocks of both meat- and dairy-type ewes. In dairy flocks, the genus has been isolated at low frequencies (0.4% in González-Rodríguez et al., 1995; 7.3% in Spanu et al., 2011). Low frequencies have also been reported in meat-type ewes with subclinical or clinical

mastitis (0.6% in Watson et al., 1990; 0.6% in Mørk et al., 2007; 3.9% in Arsenault et al., 2008).

Klebsiella spp. are facultative anaerobic organisms that belong to the Proteobacteria phylum. The bacteria are rod shaped, generally 0.5 x 0.6-6.0 µm, and occur singly, in pairs, or in short chains (Singleton and Sainsbury, 2001). Bacteria enter the mammary gland via contaminated environmental sources, often bedding (Quinn et al., 2011). Lafi et al. (1998) isolated *Klebsiella* spp. in 2.8% and 7.3% of clinical and subclinical dairy ewes, respectively. However, *K. pneumoniae* was isolated in few cases of clinical (0.4% in Mørk et al., 2007) and subclinical mastitis (3.3% in Arsenault et al., 2008) in meat-type ewes.

Pasteurella spp. belong to the phylum Proteobacteria and are facultative anaerobes (Singleton and Sainsbury, 2001). They are usually coccoid, rod shaped, or pleomorphic, range in size from 0.5 x 1-2 µm, occur singly, in pairs, or in short chains, and can be commensal or pathogenic (Singleton and Sainsbury, 2001). *Pasteurella* spp. are commonly found on mucosal linings, and their lifespan outside of these environments is short (Quinn et al., 2001). While *Pasteurella* spp. have been identified at relatively moderate frequencies in some cases of clinical and subclinical mastitis in dairy ewes (3.7-14.3%; Al-Majali and Jawabreh, 2003; Lafi et al., 1998), they are not commonly isolated in meat-type ewes. For example, Watson et al. (1990), Watkins et al. (1991) and Mørk et al. (2007) reported *Pasteurella* spp. in less than 3% of positive samples from ewes rearing their lambs.

Proteus spp. are members of the Proteobacteria phylum that are facultative anaerobic organisms (Singleton and Sainsbury, 2001). They are rod shaped bacteria, generally 0.5 x 1-3 µm (Singleton and Sainsbury, 2001). They have been isolated in low frequencies in Awassi dairy ewes (3.6 to 5.6%; Lafi et al., 1998; Al-Majali and Jawabreh, 2003), but were not been reported in the reviewed literature investigating meat-type ewes.

Pseudomonas spp. are aerobic organisms that belong to the phylum Proteobacteria (Singleton and Sainsbury, 2001). Bacteria are pathogenic, rod shaped, generally 0.5 x 1.5-5.0 µm, and found in both soil and aquatic environments (Singleton and Sainsbury, 2001). Species have been isolated at low frequencies in dairy ewes, including 2.8% and 0.3- 6.1% in clinically or subclinically infected ewes, respectively (Lafi et al., 1998; González-Rodríguez et al., 1995). The genus has also been isolated in subclinically infected meat-type crossbred ewes in Canada (4.2%; Arsenault et al., 2008).

In conclusion, there are many Gram-negative bacteria which have been isolated in cases of ovine IMI, with large variation across flocks and breeds. However, the most commonly isolated Gram-negative bacteria include *Escherichia* spp. and *Mannheimia* spp., and less common pathogens include *Acinetobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Pasteurella* spp., *Proteus* spp., and *Pseudomonas* spp. Additionally, both Gram-positive and Gram-negative bacteria frequencies reported previously are widely variable across studies, breeds, and flocks which may be attributed to different management practices and environments.

Somatic Cells

Somatic cells in milk are primarily leukocytes produced by the immune system in response to inflammation. Previous research of ovine milk identified somatic cells in both colostrum and milk collected during mid-lactation and involution (Lee and Outteridge, 1981). These researchers determined that somatic cells are primarily polymorphonuclear leukocytes, macrophages, and lymphocytes produced by the immune system in response to an inflammatory stimulus, with low frequencies of plasma and ductal epithelial cells.

Polymorphonuclear leukocytes are a type of white blood cell produced in response to inflammatory stimuli and include neutrophils, eosinophils, and basophils (Singleton and Sainsbury, 2001). These cells are important for a host's defense to recognize, ingest, and destroy pathogenic bacteria (Quinn et al., 2011). Lymphocytes, including B- and T-cells, are also white blood cells and are essential for humoral and cell-mediated immunity (Singleton and Sainsbury, 2001). Macrophages are long-lived, amoeboid phagocytic white blood cells and have important roles in host resistance to pathogenic microorganisms (Singleton and Sainsbury, 2001). The number and concentration of somatic cells in milk increases in response to infection. Therefore, SCC can be used to infer a ewe's intramammary infection status and this methodology is reviewed in the following section.

Direct Methods to Determine SCC

The determination of SCC in milk is an important part of mastitis research. There are several differences in the methods of determining SCC. In the literature reviewed, the

Fossomatic and Coulter Counters along with Direct Microscopic Cell Counts were the most prevalent methods of directly determining SCC. However, additional direct and indirect methods of quantifying SCC have been developed and will be reviewed.

Direct Microscopic Somatic Cell Count Direct Microscopic Somatic Cell Count (DMSCC) was developed by modifying and improving the Breed technique of determining the number of epithelial cells and leukocytes in milk (Prescott and Breed, 1910; Smith, 1969). Protocols for DMSCC involve preparing milk films on a glass slide. The milk films are then stained, and the slides are examined under an oil-immersion objective microscope at 930-1000 x magnification. An eye piece reticle is placed on the oculars and has parallel lines which serve as boundaries for counting somatic cells. The film is then manually scanned and cells, including leukocytes and epithelial cells whose nuclei can be distinctly stained, are counted (National Mastitis Council Subcommittee on Screening Tests, 1968). This methodology is low-throughput and requires operators to be trained to differentiate between somatic cells and non-nucleated cells. Nevertheless, it is accurate and usually regarded as the standard to which other methodologies are compared.

Coulter Electronic Counter The Coulter electronic particle counter was designed to rapidly and accurately count cells and has the capability of differentiating between leukocytes and fat globules (Phipps, 1965; Phipps and Newbould, 1966). Milk samples are first diluted with NaCl and mixed before being dispensed into a centrifuge tube. After centrifugation, milk components are separated such that cells are sedimented and fat

globules larger than 3 μm in diameter are at the surface. The sedimented cells are isolated and redispersed, and the suspension is decanted into a beaker (Phipps, 1965; Phipps and Newbould, 1966). The Coulter electronic counter then quantifies all particles of the calibrated size and larger to estimate SCC (Phipps and Newbould, 1966).

Fossomatic Somatic Cell Counter The Fossomatic somatic cell counter (Foss Electric, Hillerod, Denmark) is a fully automatic electronic instrument which uses a flouro-optical quantitative technique to determine SCC. The system works by diluting milk with a buffer and staining it with ethidium bromide. After heating to 60ºC and stirring, the stain penetrates the cell to form a complex with cellular DNA which emits a strong fluorescence under the instrument's xenon lamp. Images of the fluorescing cells are focused through a microscope onto a slit of the photo-multiplier's shielding. Each beam of light passes the slit to activate the photo-multiplier, which transmits pulses to a digital counter. The total number of impulses is then interpreted as the estimated SCC (Schmidt Madsen, 1975).

Comparison of Direct Methods to Determine SCC

As previously mentioned, DMSCC is often referred to as the "gold standard" method but requires more specialized training and is low-throughput. The Fossomatic and Coulter methods enable laboratories to estimate SCC on a large number of samples, but their accuracy is dependent on their agreement with DMSCC. Both Heald et al. (1977) and Dulin et al. (1982) reported that SCC estimated by the Fossomatic Counter was comparable to DMSCC. Estimated correlation coefficients were 0.99 when cow milk

samples were stored from 0 to 2 days and 0.78 when stored for 5 days (Heald et al., 1977). Similar results for cow milk were found by Schmidt Madsen (1975; r^2 = 0.92-0.99) and for dairy ewe milk by Gonzalo et al. (1993; r^2 = 0.99). Studies have shown the Fossomatic somatic cell counter has lower coefficient of variations in both cow (Heeschen, 1975; Heald et al., 1977) and ewe milk (Gonzalo et al., 1993) than DMSCC methods. Past research has also estimated strong positive correlation between the Coulter Counter and DMSCC methods in cow milk (r^2 = 0.89-0.98; Phipps and Newbould, 1966; Schmidt Madsen, 1975).

A few experiments comparing the Coulter and Fossomatic methods have reported the Coulter Counter estimated greater mean SCC (between 1.08 and 6.82 times greater) than the Fossomatic Somatic Cell Counter in dairy cows (Miller et al., 1986), meat-type ewes (Burriel, 2000), and dairy goats (Dulin et al., 1982). Both Burriel (2000) and Dulin et al. (1982) attributed this difference to the Coulter Counter being unable to differentiate between cells and non-cells including both cytoplasmic bodies and fat globules. Still, Green (1984) estimated strong positive correlation coefficients between Coulter and Fossomatic counters (r^2 = 0.95-0.99). Furthermore, Heeschen (1975) conducted a 3-way evaluation of DMSCC, the Coulter counter, and the Fossomatic counter and estimated similar geometric mean SCC for each method and strong positive correlation coefficients between methods (r^2 = 0.95-0.98). In conclusion, many methods have been developed to determine SCC, and past research has indicated strong correlations and agreeability between each.

Indirect Methods to Determine SCC

California Mastitis Test

The California Mastitis Test (CMT) was developed to be an on-farm method to indirectly measure SCC using the premises of the Whiteside phenomenon (Whiteside, 1939) and modified Whiteside test (Murphy and Hanson, 1941; Schalm and Noorlander, 1957). During development of the testing procedure, it was found that reagents containing Na or K salts formed a gel or precipitate when brought into contact with a high SCC milk sample. In addition, an anionic surface-active near a neutral pH (bromocresol purple; dibromo-o-cresol sulfone phthalein) was sought to serve as an indicator to distinguish alkaline or acidic milk with a contrasting color from normal milk. The procedure for on-site CMT requires foremilk samples be drawn into separate wells on a reusable plastic paddle. The reagent is then added at a 1:1 ratio and mixed with a gentle swirling of the paddle to generate a reaction. Based on physical characteristics of the reaction, a score is given to each milk sample (Table 1; Schalm and Noorlander, 1957). As the CMT score increases, so does the total cell count and percentage of polymorphonuclear leukocytes. Overall, the CMT is fast, on-farm method that is inexpensive and requires little training to become proficient.

Table 1. California Mastitis Test (CMT) scores and associated characteristics adapted from Schalm and Noorlander (1957).

CMT Score	Characteristics
Negative (-)	Mixture remains liquid with no evidence of a precipitate.
Trace (T)	A slight precipitate of the mixture is seen which tends to disappear as the paddle continues to swirl.
Weak Positive (1)	A distinct precipitate forms without the tendency to gel.
Distinct Positive (2)	The mixture thickens immediately with some gel formation, which, as swirled, moves toward the center leaving the bottom outer edge exposed until swirling ceases and mixture self-levels.
Strong Positive (3)	A distinct gel forms which tends to adhere to the bottom of the well and form a peak during swirling.
Alkaline Milk (+)	The mixture is distinctly purple. The plus sign is added to the CMT score.
Acidic Milk (y)	The mixture is distinctly yellow. The letter "y" is added to the score.

Electrical Conductivity

Technologies utilizing electrical conductivity (EC) to detect mastitis are more recent developments, though early studies discovered the underlying methods (Davis, 1947). The measurement of EC machines reflects the total ionic content of a milk sample. The major and minor constituents of the ions include Cl, Na, and K and Ca, Mg, and phosphate, respectively (Fernando et al., 1985). During development, accuracy of experimental EC results were compared to tests for chemical abnormalities in milk (rennet, chloride, and bromocresol purple paper tests) or increased cell content (cell count and centrifuged deposit, catalase, and resazurin tests). Today's on-farm technology that utilizes principles of EC generally consist of a milking-cup, carrying arm, and a meter that displays chloride percentages and conductivity. A foremilk sample is directed into

the milking cup, the device is operated, and the conductivity is displayed. Devices are self-cleaning, so samples can rapidly be measured.

Comparison of Indirect Methods to Direct Methods

Heald et al. (1977) compared dairy cattle CMT scores to estimated SCC derived from a Fossomatic counter. Fossomatic SCC was first converted to CMT score as: 0 to 200 x 10^3 cells/mL = N; 201 x 10^3 to 400 x 10^3 cells/mL = T; 401 x 10^3 to 800 x 10^3 cells/mL = 1; 801 x 10^3 to 5000 x 10^3 cells/mL = 2; and > 5000 x 10^3 cells/mL = 3. Mean Fossomatic SCC for a CMT score of N and T were 171 x 10^3 and 439 x 10^3 cells/mL, respectively. Furthermore, the converted Fossomatic and actual CMT score showed 68 and 49% agreeability, respectively. For CMT scores of 1, 2, or 3, mean SCC were 750 x 10^3, 1354 x 10^3, and 2546 x 10^3cells/mL with 55, 36, and 16% agreeability between SCC and predicted CMT score, respectively (Heald et al., 1977). The lower agreement between SCC and CMT scores 2 and 3 was attributed to reduced sample sizes. Maisi et al. (1987) measured the correlation between SCC and CMT to be 0.69 in Manchega ewes.

Fernando et al. (1985) compared efficacies of various methods of detecting SCM, including EC, SCC and concentrations of chloride, sodium, potassium, lactose, and bovine serum albumin. The study provided little evidence of a correlation between EC and log-SCC (r^2 = 0.21). An earlier study showed the number of quarters with elevated conductivity tended to be higher among quarters with SCC greater than 500 x 10^3 cells/mL in both uninfected and infected groups, although agreement was low (16.7%;

Fernando et al., 1980). However, Peris et al. (1991) estimated moderate to high correlations between SCC and CMT (0.64-0.68), SCC and EC (0.52-0.53), and CMT and EC (0.74-0.76) in foremilk and stripping samples from ewes.

Somatic Cell Count Thresholds Used to Diagnose Subclinical Mastitis

To maintain high quality dairy products and protect human health, the legal maximum bulk-tank SCC in the U.S. is 750,000 cells/mL (HHS, 2015), regardless of specie. Regulatory SCC thresholds are adapted from multiple investigations where researchers attempt to diagnose subclinical mastitis in individual animals. While SCC can be quantified rapidly and economically, its ability to classify the IMI status of an individual is not without error and has been the focus of many studies. The design of these experiments involves jointly quantifying SCC and bacteriological status of milk samples from clinically healthy animals. Somatic cell count thresholds are then evaluated on their efficacy of correctly identifying an animal's bacteriological status. Several classification statistics can then be estimated and are visualized below:

	Bacteriological status	
SCC test$_k$	Positive	Negative
Positive	n_{11}	n_{12}
Negative	n_{21}	n_{22}

Here, bacteriological status (positive/negative) of the milk sample is the reference method and whether its SCC is greater than (positive) or less than (negative) threshold *k* (e.g.,

200 x 10^3 cells/mL) is the alternative method. The n_{ij} represent the number of samples in each category. The most common statistics estimated in these studies include sensitivity, specificity, positive predictive value, and negative predictive value.

Sensitivity (Sen), or true positive rate, is the proportion of reference method positive samples that has been classified as positive by the alternative method (n_{11}/[n_{11} + n_{21}]). Specificity (Spe), or true negative rate, is the proportion of reference method negative samples that has been classified as negative by the alternative method (n_{22}/[n_{22}+n_{12}]). Positive predictive value (PPV), or precision, is the proportion of reference method positive samples relative to the total number of positive samples predicted by the alternative method (n_{11}/[n_{11} + n_{12}]). Negative predictive value (NPV) is the proportion of reference method negative samples relative to the total number of negative samples predicted by the alternative method (n_{21}/[n_{21} + n_{22}]). In the literature, these statistics are generally expressed as a percentage.

Malek dos Reis et al. (2011) evaluated SCC thresholds to predict bacteriological status for major pathogens in individual cow quarters. Both Sen (74 to 45.4%) and NPV (86.6 to 80.8%) decreased while Spe (59.1 to 81.2%) and PPV (39 to 46%) increased as the SCC threshold increased from 100 x 10^3 to 400 x 10^3 cells/mL. In an earlier study, Dohoo and Leslie (1991) evaluated SCC thresholds and found Sen decreased (84.3 to 71.7%) and Spe increased (85.5 to 96.2%) as the threshold increased from 200 x 10^3 to 250 x 10^3 cells/mL. Additionally, Dohoo et al. (1981) estimated Sen to be 86% at a SCC of 228 x 10^3 cells/mL. These results generally agree with others that have proposed SCC

thresholds in dairy cattle: 100 x 10^3 (Schwarz et al., 2010), 250 x 10^3 (Berry and Meaney, 2006), and 300 x 10^3 cells/mL (Deluyker et al., 2005).

Unlike dairy cattle, past research in dairy ewes has not reached such a narrow range of SCC thresholds corresponding to IMI. González-Rodríguez et al. (1995) cultured milk samples from separate halves of Assaf, Churra, and Castellana dairy ewes across 18 flocks in Spain and quantified SCC via a Fossomatic methods. Researchers then evaluated a range of SCC thresholds (100 x 10^3 to 500 x 10^3 cells/mL) and calculated Sen and Spe at each. It was determined that 300 x 10^3 cells/mL was optimal and corresponded to Sen and Spe values of 79.5 and 82.2%, respectively. Riggio et al. (2013) sampled Valle del Belice ewes in four flocks at approximately 1-mo intervals throughout lactation. Fossomatic SCC was quantified and used to predict IMI status of major and minor pathogens identified in culture. When including all pathogens, the optimal SCC threshold was 645 x 10^3 cells/mL, which yielded Sen, Spe, PPV, and NPV values of 53, 86, 69, and 76%, respectively. The optimal SCC threshold was similar (645 x 10^3 cells/mL) when only minor pathogens were included, and resulted in Sen, Spe, PPV, and NPV values of 48, 86, 64, and 77%, respectively. When only major pathogens were used, the optimal SCC threshold was 2138 x 10^3 cells/mL, which yielded Sen, Spe, PPV, and NPV values of 61, 93, 37, and 97%, respectively. Suarez et al. (2002) measured SCC and performed bacterial culture of dairy ewe (Pampinta; ¾ East Friesian x ¼ Corriedale) milk and reported mean SCC of 375 x 10^3 and 1464 x 10^3 cells/mL in healthy and subclinically infected ewes, respectively. Furthermore, at a SCC threshold of 1200 x 10^3 cells/mL, Sen, Spe, PPV, and NPV were 73, 87, 78, and 83%, respectively.

Somatic cell count thresholds to infer IMI status have also been examined in non-dairy ewes. Clements et al. (2003) collected SCC and bacteriological data from milk samples from Scottish mule ewes. The point where Sen was equal to Spe (67%) corresponded to a SCC threshold of 1284 x 10^3 cells/mL. Świderek et al. (2016) collected milk samples from Polish Heath and Lowland ewes, quantified SCC by flow cytometry, and identified microorganisms using a commercial test kit. At a SCC threshold of 205 x 10^3 cells/ mL, Sen and Spe were 74 and 66%, respectively. Maisi et al. (1987) collected milk samples from Finnsheep, Texel, and their crossbreds and concluded a SCC threshold of 1660 x 10^3 cells/mL was optimal to diagnose subclinical mastitis with a Spe of 81.6%.

The previous studies estimated the ability of SCC thresholds to accurately diagnose IMI in naturally infected populations of ewes. Potential explanations for observed variability of recommended SCC threshold among studies may include breed differences in immune response, animal purpose or production system (e.g., dairy, meat, or fine-wool), and causative pathogens. To account for some of these extraneous variables, Fthenakis et al. (1991) inoculated Dorset Horn and Welsh Mountain ewes with *S. simulans* 6 or 16 d postpartum to induce subclinical mastitis in two separate experiments and compared SCC response with control ewes. Milk SCC showed a threshold of 1000 x 10^3 cells/mL was indicative of mastitis, as 98.2% of control ewes had a SCC less than and 85.8% of inoculated ewes had a SCC greater than that threshold.

Prevalence of Ovine Clinical and Subclinical Mastitis in Non-Dairy Flocks

Mastitis is a globally prevalent disease, and many researchers have estimated the frequency of clinical and subclinical mastitis across multiple flocks or within a single flock. Early research conducted across New Zealand Romney flocks estimated the average incidence of clinical mastitis to be 1.1-2.3% (Quinlivan, 1968). Koop et al. (2010) reported an incidence of clinical mastitis in a flock of Texel ewes in the Netherlands to be between 7 and 9%. Watson et al. (1990) reported that glands which presented clinical mastitis had infection rates between 10 and 29% across 8 flocks in Australia. Recently, Grant et al. (2016) estimated a 2-3% incidence acute mastitis across 10 English flocks, though considerable variability within flocks was observed (0-37%). Arsenault et al. (2008) observed a low (1.2%) incidence of clinical mastitis across 30 flocks in Canada. However, culture methods revealed a greater incidence of SCM (18%) in these flocks. This is a common theme across the reviewed literature, that is, the incidence of SCM is greater than clinical mastitis in most ewe flocks.

Watson et al. (1990) collected milk from clinically normal mammary glands across 8 Australian flocks and estimated that between 4 and 10% tested positive upon bacteriological culture. Similarly, Watkins et al. (1991) found the prevalence of SCM based on culture status to be between 6.4 and 18.9% across 7 flocks in southern England. More recently, Persson et al. (2017) reported a 30% incidence of IMI using culture methods across 22 Swedish flocks. In a study of Finnsheep and Texel ewes originating from a single Finnish flock, Maisi et al. (1987) determined the prevalence of IMI to be 16.7% after examination of bacterial cultures.

The majority of studies investigating ovine mastitis in U.S. sheep have been conducted in a single flock. Under range conditions in Montana, Marsh (1958) reported that, over the course of 10 years and covering 19,550 ewes bred, the incidence of clinical mastitis was 2.3% in ewes managed at the Montana Experiment Station. In a research flock in California, Targhee ewes were sampled over two production years and CMT scores (bilateral N or T score; unilateral 3, 4, or 5 score; bilateral 3, 4, or 5 score) indicated that 8.2 and 7.3% of all ewes were subclinically infected at lambing or docking, respectively (Gross et al., 1978). Torres-Hernandez and Hohenboken (1979) also used CMT to infer IMI in a research flock of crossbred range-type ewes in Oregon and reported an 11.5% overall incidence of mastitis. Kirk et al. (1980) sampled a single range flock in Idaho, and bacterial culture revealed a clinical mastitis incidence of 2.6%. Furthermore, the frequency of SCM was 47.4 and 30.2% at parturition and 3 wk postpartum, respectively. In an Ohio flock (Dorset, Hampshire, Suffolk, Rambouillet, and crossbred ewes), Hueston et al. (1980) reported a culture-positive incidence rate of 4.2 and 16.7% for clinical and subclinical mastitis, respectively. Later, Hueston et al. (1986) reported the frequency of SCM in udder halves to range between 10.4 (2 wk postpartum) and 29.0% (3 wk postweaning) in the same flock. McCarthy et al. (1988) used CMT to diagnose SCM (negative = 1; positive = 2; strong positive = 3) in a three-year evaluation of a single flock in Virginia. Milk was collected from study ewes at parturition, 3 wk postpartum, and at weaning, and SCM incidence rates across breeds ranged from 27.3 to 40.7%, 14.3 to 60.0%, and 18.2 to 60% at these time points, respectively. Across three flocks in Iowa, the incidence of SCM based on culture methods was 24.5 and 21.9% at

lambing and weaning, respectively (Ahmad et al., 1992a). Finally, Keisler et al. (1992) estimated a SCM frequency between 11 and 27% in a research flock of Hampshire and crossbred ewes in Missouri using CMT methodology. In summary, past reports have used both culture and SCC methodology to infer clinical and subclinical mastitis and their frequency is variable across flocks. Nevertheless, mastitis has been reported in both extensively and intensively managed flocks and its impacts on ewe and lamb welfare and productivity will be reviewed in the following sections.

Production Impacts of Mastitis

Subclinical mastitis is estimated to cost the U.S. dairy cattle industry more than $1 billion dollars annually due to production losses (Ott, 1999). A thorough analysis of the economic impact of mastitis on the U.S. sheep industry has not been completed, though earlier worked estimated an annual cost of $20 to $25 million (Smith, 1989; Ahmad et al., 1992a). Outside the U.S., assuming a conservative 10% incidence of SCM in EU dairy sheep flocks and goat herds, it has been estimated that losses could be €60 million ($67.39 million) per year (Rupp and Foucras, 2010). The economic impact of mastitis in meat-type sheep may not be as large as in dairy ewes, however, if the risk of contracting clinical mastitis could be reduced by 10%, Conington et al. (2008) estimated an additional yearly revenue of £2.7 million ($3.51 million) to the purebred Texel industry within the UK. Common financial losses attributed to ovine clinical mastitis are ewe culling/death, lamb morbidity/mortality, and costs associated with replacement ewes,

labor, milk replacer, and veterinary treatment (Timms, 2007). However, since diagnosis of SCM does not occur in most non-dairy flocks, its economic impact is not clear.

Ewe Culling and Mortality

Clinically infected ewes require treatment and should be culled, and both practices reduce profitability of sheep enterprises. However, in the worst case scenario, clinically infected ewes may die and record no salvage value. In a survey of Canadian sheep producers, mastitis was reported to be the fourth common reason of both ewe morbidity (2.1%) and mortality (0.2%), and the single most important reason for involuntary culling (15.2%; Dohoo et al., 1985). Waage and Vatn (2008) determined that ewes which have had clinical mastitis in a previous lactation are 4 times more likely to experience subsequent infection or lose the function of one or both glands. Considering clinical cases in a purebred U.S. research flock of non-dairy ewes, Hueston et al. (1980) calculated a mortality rate of 42.9%. However, Arsenault et al. (2008) reported 2% of clinically infected meat-type ewes were culled without administering of antibiotics. In a survey of slaughter ewes sampled at an UK abattoir, 50.2% of udders were classified as abnormal, although researchers couldn't conclude this to be the primary reason for premature culling (Herrtage et al., 1974). Similarly, Watson and Buswell (1984) reported that udder abnormalities, particularly those associated with infection, accounted for nearly half (46%) of all cull ewes in a typical lowland flock in the UK. In the U.S., a more recent USDA survey found mastitis accounted for 7.6% of ewes culled (USDA APHIS, 2011). Therefore, mastitis and udder abnormalities are a globally common reason to cull ewes prior to the end of their normal productive life.

Lamb Mortality and Performance

Lambs reared by dams with mastitis can suffer from enhanced mortality and reduced performance. In a case study, Holmøy et al. (2014) reported that 6.7 and 10.4% ewes listed in a Norwegian registry with mild or moderate to severe clinical mastitis lost lambs within 5 days of lambing, respectively. Another survey conducted in Scotland reported 34.1% of lamb losses were attributed to starvation (Johnston et al., 1980), which was partially attributed to the dam having an insufficient milk supply. However, Kirk et al. (1980) found no relationship between dam IMI and litter survival in a shed-lambed, range flock in Idaho. Still, Christley et al. (2003) determined that dam clinical mastitis was associated with reduced lamb serum immunoglobulin concentration and greater lamb mortality.

The relationship between milk yield and SCC has been well researched in dairy (Crossman et al., 1950; Bartlett et al., 1991; Lescourret and Coulon, 1994) and beef cows (Simpson et al., 1995; Brown et al., 1996; Lents et al., 2002). In dairy sheep, past research has reported a 2.6-54.1% reduction in milk yield accompanying SCM and is dependent upon the type of bacteria, flock, and breed (Dario et al., 1996; Saratsis et al., 1999; Winter et al., 2003). Studies of meat-type ewes with SCM acquired both experimentally (Fthenakis and Jones, 1990b) and naturally (Torres-Hernandez and Hohenboken, 1979; McCarthy et al., 1988; Moroni et al., 2007) have estimated an 11-58% reduction in milk yield. Furthermore, milk from subclinically infected ewes has increased protein and decreased lactose and fat content (Torres-Hernandez and Hohenboken, 1979; McCarthy et al., 1988). These researchers attributed protein

differences to an additional protein in the inflammatory and immune responses or elevated white blood cell concentration. A cause-and-effect relationship could not be established between lactose or fat composition and mastitis. McCarthy et al. (1988) attributed milk yield reductions to a decrease in functional mammary tissue but noted it may be related to a reduction in lactose production.

Because of the reduction in milk quantity and quality accompanying SCM and past reports of a positive association between dam milk production and lamb growth (Barnicoat et al., 1949; Burris and Baugus, 1955, Torres-Hernandez and Hohenboken, 1979), it's a natural progression to associate dam SCM with reduced lamb growth. However, this relationship is difficult to estimate in practice because lambs can obtain nutrients from a variety of sources besides their dam's milk. Even when reared by healthy ewes, lambs have been shown to nurse other dams (Hess et al., 1974). Additionally, many conventional lambing systems offer lambs free choice creep feed within a few days of being born. For these reasons, the direct relationship between dam milk SCC and lamb performance has been insignificant (Hueston, 1980; Keisler et al., 1992) or inconsistent (Ahmad et al., 1992a). Both Gross et al. (1978) and Ahmad et al. (1992a) reported reductions of ADG by between 2.6 and 4.8 kg or 4.6 to 11.3%, respectively, in lambs reared by infected ewes. Fthenakis and Jones (1990b) were able to quantify growth and creep feed consumption and reported that lambs reared by inoculated ewes weighed between 9 and 40% less at 52 d postpartum and consumed 28% more creep feed than lambs reared by healthy ewes (Fthenakis and Jones, 1990b).

Husbandry Practices to Reduce the Incidence of Mastitis in Ewes

Mammary Recovery and Post-Weaning Fasting of Ewes

Two to three weeks following weaning (meat and wool) and dry-off (dairy) has been associated with a 13-80% increased risk of IMI in ewes (Hueston et al., 1986; Saratsis et al., 1998). The period from when lambs are weaned and cessation of lactation has begun until the subsequent parturition event is known as the dry-period. The dry-period is characterized by three phases: (i) active involution, (ii) steady-state involution, and (iii) redevelopment and lactogenesis (Petridis and Fthenakis, 2014). Involution is the process of mammary tissue returning to a non-secreting state (Petridis et al., 2013). Proper management of flock mammary health, including curing previous IMI and preventing new ones, culling ewes unsuitable for lactation, and maintaining strict hygienic standards during the dry-period optimizes milk production in subsequent lactations (Contreras et al., 2007; Fthenakis et al., 2012). Still, general weaning practices aimed at reducing IMI in non-dairy flocks are not well researched.

A commonly recommended post-weaning husbandry practice is to withhold feed from ewes for 48 h followed by feeding a low-energy, low-protein diet for the next 10-14 d (SID Sheep Production Handbook, 2002). This practice is thought to help terminate lactation and begin involution of the mammary tissue. However, few research reports have evaluated its effectiveness. McCarthy et al. (1988) evaluated the effect of a 72 h post-weaning fast in an accelerated lambing system on mastitis incidence in the subsequent lactation. They reported no effect of fasting on the frequency of positive CMT in the next lactation, however fasted ewes had reduced udder size and swelling for

several days following weaning. Additionally, ewes that were fasted after weaning during two consecutive yr produced 23.4% more milk 21 d after parturition the following yr.

Antibiotic Therapies

Administering antibiotics to females prior to, during, or after lactation can be a therapeutic and prophylactic treatment against IMI (Postle and Natzke, 1974; Fthenakis et al., 2012). Dry-off treatment is the administration of a routine long-acting antibiotic into each teat canal at the end of lactation and has been recommended to control mastitis in dairy cows for over 50 years (Neave et al., 1966). The effectiveness of antimicrobial dry-off treatment in dairy sheep has not been documented as long, though reports have indicated that ewes provided with treatment exhibited 6-10 times greater cure rates and reduced new infection rates by 1.6-2.9 times (Chaffer et al., 2003; Linage and Gonzalo, 2008). Furthermore, researchers have reported other positive effects of antibiotic therapies in dairy sheep, including reducing IMI frequency by 2.1 times, increasing milk yield by 6.9%, and reducing SCC in subsequent lactations by 17-34% (Gonzalo et al., 2004; Linage and Gonzalo, 2008; Spanu et al., 2011). Gonzalo et al. (2004) reported that the prevalence of IMI in dairy ewes at dry-off decreased when the entire flock is treated with an antibiotic (32%) compared to animals not treated (65%). There are fewer studies that have evaluated the efficacy of antibiotic treatments at dry-off in non-dairy ewes. In an early study, Buswell and Yeoman (1976) infused both teats of Scottish Cheviot ewes (n ~700) with cloxacillin at weaning. Though this study lacked an adequate control treatment, only two cases of clinical mastitis were observed during the ensuing dry period. In a study of 931 ewes across three flocks in the UK, 462 ewes were infused with

a dry-cow cerate (1 g procaine penicillin + 0.5 g dihydrostreptomycin sulphate) in each udder half when their litters were weaned. Treated ewes had a reduced frequency of clinical mastitis at breeding (1.5%) compared to the control group (4.5%; Hendy et al., 1981). McCarthy et al. (1988) treated ewes with an intramuscular injection of penicillin at weaning, and reported increased milk yields of 14.3% into the third year of the study and a reduction of the incidence of subclinical mastitis by 57% at weaning during the second year of the study. A study evaluating the efficacy of intramammary infusion with cephapirin benzathine at weaning reported untreated ewes were 2.6 times more likely to develop an IMI between sampling times (Hueston et al., 1989). Similarly, Ahmad et al. (1992b) reported that injections of cephapirin benzathine (intramammary) or benzathine penicillin (intramuscular) at weaning resulted in 2 times greater cure rates and reduced new infection rate by 8.3-23.8%. Another study administered tilmicosin to ewes 30 d prior to lambing and observed a 43% decrease in palpable udder abnormalities, but no reduction in clinical mastitis compared to untreated ewes (Croft et al., 2000). However, lambs from treated ewes were 2.6% heavier (0.52 kg) at 50 d than lambs reared by control ewes. In summary, antibiotic treatments have been shown to improve mammary health in ewes, though the cost-effectiveness of doing so has not been evaluated.

Genetic Selection

Small ruminant research pertaining to mastitis genetics and selection has been growing recently, of which the majority has concentrated on dairy ewes. Murphy et al. (2017a,b) estimated the heritability and repeatability for average lactation somatic cell score to be 0.12 and 0.31, respectively, in a research flock of crossbred dairy ewes in

Wisconsin. Similarly, Hamann et al. (2004) estimated heritability and repeatability for somatic cell score in German East Friesian ewes to be 0.16 and 0.24, respectively. Similar heritability estimates for SCC measures in dairy sheep have been estimated by others (0.12 to 015; El-Saied et al., 1999; Barillet et al., 2001; Rupp et al., 2003). Fewer genetic evaluations for mastitis indicators have been reported in non-dairy ewes. McLaren et al. (2018) estimated heritabilities for average somatic cell score to be 0.11 and 0.08 for mid- and late-lactation, respectively, in UK Texel flocks. Additionally, heritability estimates for CMT were between 0.07 and 0.11. In Texel ewes across 10 registered flocks in the UK, Crump et al. (2018) estimated the mean heritability of chronic mastitis to be 0.09. Therefore, the heritabilities of clinical and subclinical mastitis are low, thus, genetic improvement in these traits is likely to be lower than many other commercially important traits.

Udder and Teat Characteristics

Intramammary Masses

Intramammary masses (IMM) are defined as physically detectable lumps of abnormal consistency compared with the rest of the mammary tissue. Grant et al. (2016) reported factors related to IMM include mastitis, teat lesions, udder conformation, previous IMM during pregnancy or lactation, and underfeeding energy or protein requirements during lactation. Studies often categorize IMM as present/absent though individual cases can vary in degree. Frequencies of IMM have been largely variable across flocks and studies. In a study of 10 farms of Lleyn, Charollais, Texel, and

crossbred ewes in the UK, researchers determined that 4.1-8.7% of ewes have an IMM during pregnancy and 5.7-14.3% have an IMM during mid-lactation (Grant et al., 2016). Similarly, Griffiths et al. (2019a) reported an IMM rate of 3.0-5.9% throughout the production year in Romney ewes in a single New Zealand flock. However, Crump et al. (2018) calculated a much greater frequency of 30% in Texel ewes across 10 flocks in the UK. Petridis and Fthenakis (2014) suggested IMM are a common reason for culling ewes. Herrtage et al. (1974) found abnormal udders, including IMM, in 50.2% of cull ewes, although this estimate included other abnormalities such as teat lesions. Similarly, Madel (1981) determined palpable masses were found in 10.4% of cull ewes at an abattoir in England.

Researchers have also assessed the relationship between IMM and mastitis or lamb performance. Grant et al. (2016) calculated the odds of acute mastitis are 1.82 times and 3.16 times greater for ewes which experienced an IMM during pregnancy or previous lactation, respectively. Griffiths et al. (2019a) calculated the odds of failure of a lamb to survive until weaning increased 1.6-4.0 times when reared by dams with IMM. Similarly, average growth rates of these lambs were reduced by 0.4-10.9%. In conclusion, palpable masses in mammary tissue are common in ewes and have been shown to be detrimental to udder health and lamb growth.

Teat Lesions

In sheep, teat lesions such as bite wounds, tears, or chapping can be infectious or non-infectious and have been reported in several studies. In slaughter ewes at abattoirs across Northern Iraq, 3.9% had mammary lesions, which, in part, included mastitis and

neoplasms (Sulaiman and Al-Sadi, 1992). Similarly, Madel (1981) determined the frequency of clinically apparent lesions in cull ewes in England to be 1.3% and an additional 2.8% had lesions which were only apparent upon close observation. However, Watkins et al. (1991) reported no association between the presence of teat lesions and SCM across 7 English flocks. Similarly, Huntley et al. (2012) reported no association between presence of teat lesions and SCC. However, Grant et al. (2016) found ewes with traumatic teat lesions (e.g., broken skin) had increased odds (2.48) of IMM across 10 flocks in the UK. Additionally, Huntley et al. (2012) estimated that lambs reared by ewes with traumatic teat lesions had lighter weights (-0.91 kg) than lambs reared by ewes without teat lesions 14 d after the lesion was observed. This was attributed to ewes preventing their lamb(s) from suckling until the wound was healed. Finally, Grant et al. (2016) reported lambs reared by ewes with traumatic teat lesions had reduced ADG (-0.02 kg/d). Therefore, researchers have not identified consistent relationships between teat lesions and mastitis, although associations between lesions and IMM or reduced lamb performance have been reported.

Linear Evaluation Systems for Udder Traits

In the dairy cattle industry, researchers have developed linear scoring systems to evaluate and rank individuals on a combination of udder traits (Thompson et al., 1983; Lucas et al., 1984; Schaeffer et al., 1985). Similar scales have been developed within specific dairy sheep breeds (de la Fuente et al., 1996; Casu et al., 2006) and applied to others (Fernández et al., 1997; Serrano et al., 2002; Legarra and Ugarte, 2005). Within Churra dairy ewes, de la Fuente et al. (1996) developed linear scales for udder depth,

attachment, and shape as well as teat placement and size. Similarly, Casu et al. (2006) developed a scoring system for teat placement, udder depth, degree of separation of udder halves, and degree of suspension of the udder halves for Sardinian dairy ewes. The linear scales of de la Fuente et al. (1996) and Casu et al. (2006) are displayed in Fig. 1 and 2, respectively.

De la Fuente et al. (1996) estimated high repeatabilities within a single lactation for linearly scored udder traits, with ranges of 0.57 (udder depth) to 0.73 (teat placement). Furthermore, it was reported that each trait score decreased by 5-21% from the first to fifth month of lactation, with the exception of teat placement which did not vary across lactation. Fernández et al. (1997) applied these scales to Churra ewes and reported similar repeatabilities within a single lactation (0.51-0.64). The repeatability of udder traits defined by Casu et al. (2006) were highly repeatable both within and across lactation (0.57-0.86).

Across udder traits, de la Fuente et al. (1996) estimated strong positive phenotypic correlations between teat placement and size (0.40), teat placement and udder shape (0.69), and udder shape and udder attachment (0.53). Serrano et al. (2002) estimated udder depth and 120 d milk yield to be strongly correlated (0.64) using the same scoring system. Casu et al. (2006) estimated strong genetic correlations between teat placement and degree of suspension of the udder (-0.69), teat placement and udder depth (-0.55), and degree of suspension of the udder and udder depth (0.82).

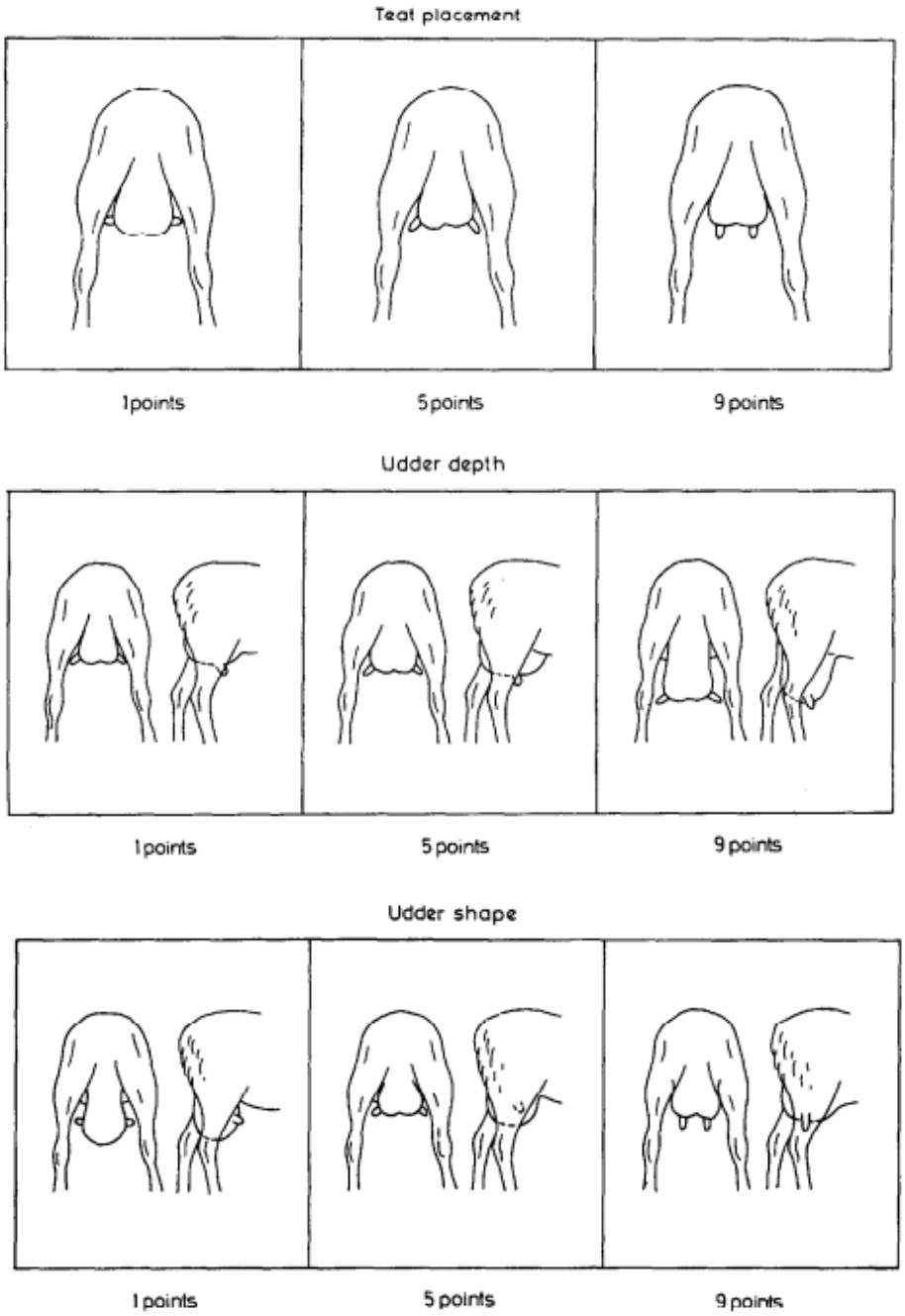

Figure 1. Linear scales for teat placement (top), udder depth (middle), and udder shape (bottom) defined by de la Fuente et al. (1996).

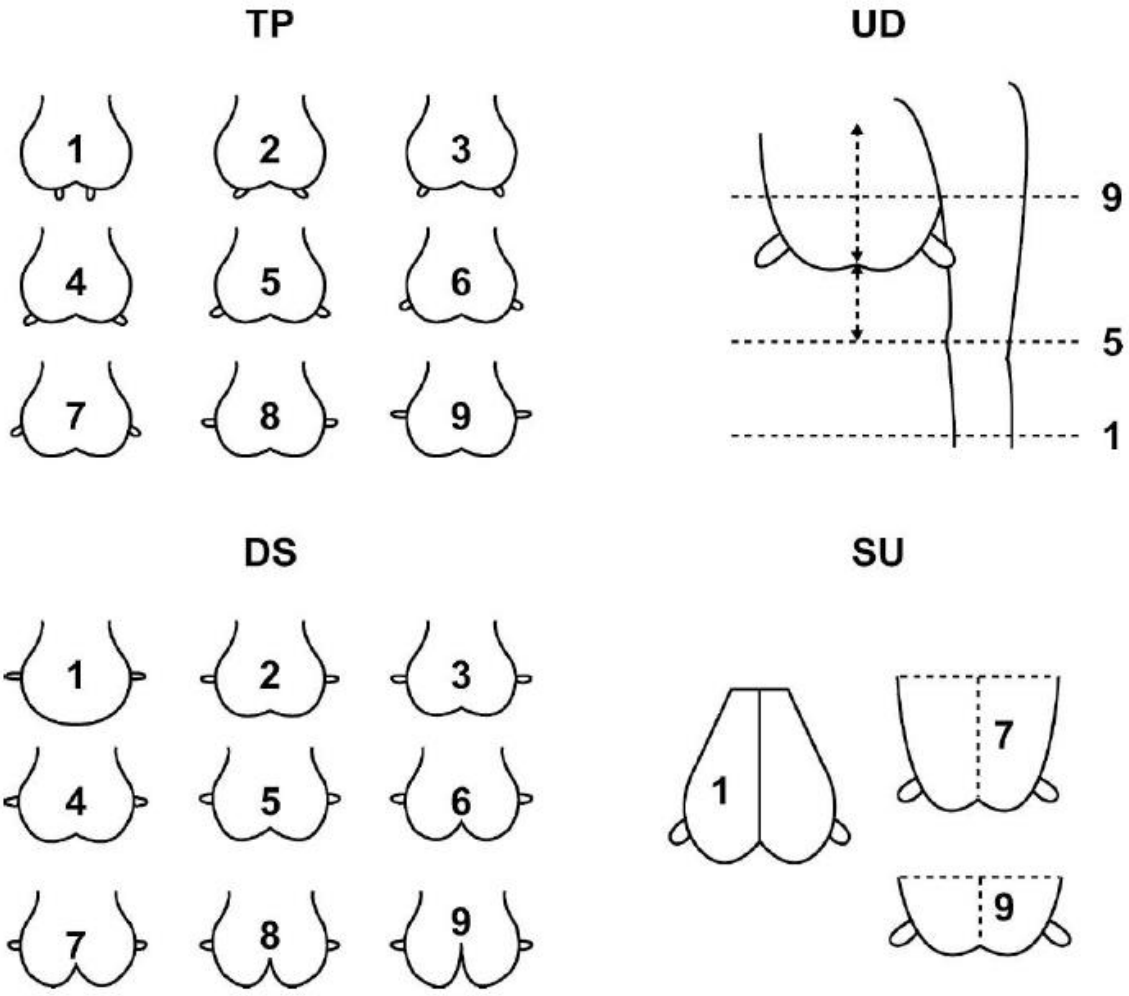

Figure 2. Linear scoring systems for teat placement (TP; top left), udder depth (UD; top right), degree of separation (DS; bottom left), and degree of suspension of the udder (SU; bottom right) defined by Casu et al. (2006).

The scoring system developed by Casu et al. (2006) has been applied to meat-type sheep as well. Cooper et al. (2013) estimated that teat placement was moderately positively correlated with both udder width (0.26) and depth (0.35) in English mule and Suffolk-cross ewes. Strong phenotypic correlations between traits were reported by McLaren et al. (2018) for udder depth and length (0.63), udder depth and width (0.45), and udder length and width (0.39). Moderate correlations were also estimated between

udder depth and teat width (0.24), udder attachment and width (0.29), udder length and teat width (0.20), teat placement and length (-0.21), and teat placement and width (-0.21).

Optimal udder and teat morphometry are likely different for dairy and non-dairy ewes. Ideal teat placement for dairy ewes are closer to a vertical position to accommodate more efficient machine or hand milking. However, in non-dairy ewes, an outward teat placement allows nursing lambs better access. For example, Huntley et al. (2012) reported lower lamb weights (-1.38 kg) were associated with suboptimal teat positions scored 2 wk post-lambing (≠ 5) using the linear scoring system developed by Casu et al. (2006).

Other investigators have developed and evaluated simpler udder scoring systems for non-dairy ewes. Griffiths et al. (2019a) reported that mature ewes with an udder depth closer to the hock prior to mating have greater odds (OR = 1.4-13.4) of not rearing lambs to weaning in their subsequent parity. Additionally, ewes with teats that are closer to the vertical prior to mating had greater odds (1.3-2.3) of not rearing a lamb that survived to weaning than ewes with teats at a 45° angle. However, ewes with deeper udders prior to mating, docking, or weaning reared lambs with up to 38% greater daily gains (Griffiths et al., 2019b). Still, this relationship was inconsistent as lambs from dams with an udder depth closer to the hock prior to parturition had 17% lower daily gains. Negative associations between udder depth and SCC have also been shown. Huntley et al. (2012) estimated that, for every increase of drop for pendulous udders, SCC increased by 9.6% (Huntley et al., 2012). Furthermore, Cooper et al. (2013) reported the odds of traumatic teat lesion are 2.6 times greater in lowland ewes with narrow or wide udder width in

suckler ewes in England, which the researchers hypothesized could be a result when lambs demand for milk is not met. In conclusion, linear scoring systems have been shown to be an effective method to analyze udders and udder halves, with strong correlations being reported for various measurements.

Additional Udder Morphometric Traits

Udder circumference and width are measured around the widest point of the udder and from the udder cleft to abdominal wall along the mid-line, respectively. In dairy ewes, these measurements have been associated with the quantity of milk produced and could explain up to 45% of milk yield variation in dairy sheep (Kominakis et al., 2009). Teat length, measured from the base of the teat to its end, is also an important morphometric trait that has implications to dairy ewe milking ability and, likely, a lamb's ability to nurse. Legarra and Ugarte (2005) estimated a strong genetic correlation for teat length across first and later lactations (0.95) in Spanish Laxta dairy ewes. Additionally, genetic correlations between teat size and milk yield (-0.10) or SCC (0.29) were weak to moderate (Legarra and Ugarte, 2005). Kominakis et al. (2009) estimated strong correlations between udder width and height (0.40), udder circumference and height (0.42), udder width and circumference (0.69), cistern depth and teat angle (0.64), teat angle and cistern depth (0.64), teat length and circumference (0.57), and teat length and angle (-0.39). Additionally, moderate correlations were estimated between cistern depth and udder height (0.32), teat circumference and udder height (0.25), cistern depth and teat length (-0.24), and teat circumference and angle (-0.23). Marie-Etancelin et al (2005) estimated udder depth to be moderately negatively correlated to milk yield and SCC (-

0.21 each) in Lacaune dairy ewes. Fernández et al. (1995) estimated phenotypic correlations between several udder measurements in Churra dairy ewes across 3 Spanish flocks. Strong correlations were estimated between udder width and depth (0.57), udder circumference and depth (0.74), udder circumference and width (0.76), teat angle and cistern depth (0.56), teat length and angle (-0.45), and teat width and angle (0.63). Moderate correlations were estimated between cistern and udder depths (0.35), cistern depth and udder width (0.20), teat angle and udder width (-0.17), teat length and udder depth (0.28), teat length and udder width (0.26), teat length and cistern depth (-0.29), teat width and cistern depth (-0.23), teat width and angle (-0.30), and teat length and udder circumference (0.28). In summary, physical measurements of udder morphometric traits have been shown to be moderately to highly correlated and associated with milk yield and SCM indicators in several studies.

Summary and Implications

In conclusion, a review of literature has revealed several areas of ovine mastitis that have been well researched and areas where it is lacking. This review has placed particular emphasis on etiology and pathogens commonly isolated in clinically and subclinically infected ewes, somatic cells and their relationship with intramammary infection, production impacts of mastitis, and morphometric characteristics of udders and teats. Considerable variation of bacteria species isolated from cases of intramammary infection across years, flocks, breeds, and stages of lactation has been documented. Somatic cells in milk can be used as an indicator of ovine subclinical mastitis, but exact

thresholds are dependent on ewe breed and management system. While phenotypic variation in ovine mastitis is mostly explained by non-additive genetic effects, important associations between udder health and udder and teat morphometric traits have been observed. Though the prevalence of clinical mastitis is low in most flocks, subclinical mastitis is quite common. Most importantly, mastitis is an important disease in sheep both from an economic and animal welfare perspective. Therefore, this disease warrants further investigations to better understand causes and preventative measures.

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CHAPTER THREE

THE PREVALENCE AND ETIOLOGY OF SUBCLINICAL MASTITIS AND ASSOCIATION WITH MILK SOMATIC CELL COUNT IN WESTERN RANGE FLOCKS

Contribution of Authors and Co-Authors

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Running head: Mastitis in sheep

The prevalence and etiology of subclinical mastitis and association with milk somatic cell count in Western range flocks[1,2]

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ABSTRACT: Mastitis in both its clinical and subclinical states is an important disease to all sheep producers, resulting in production and financial losses. However, much of the past research has been conducted in dairy ewes, so the impacts of subclinical mastitis in non-dairy ewes are less clear. The objectives of this study were to identify bacterial species present in ewe milk and evaluate somatic cell count (SCC) thresholds to predict intramammary infection (IMI) in 2 research flocks (Montana State University, MSU; U.S. Sheep Experiment Station, USSES). Milk was collected from clinically healthy ewes in early and peak lactation and used to quantify SCC and identify taxa by both Multiplex-PCR (16-plex) and culture followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Binomial proportions were estimated within culture- and PCR- positive samples. Across flocks, 63

and 43% of ewes were culture-positive for any bacteria and 25 and 30% were positive within the PCR test screen at early and peak lactation, respectively. Sixty unique classifications at either the species or genera level were identified by MALDI-TOF-MS. *Bacillus licheniformis* (20%), *Staphylococcus epidermidis* (14%), and *B. altitudinis* (12%) were the most common across flocks in early lactation. At peak lactation, *B. licheniformis* (14%) was most common across flocks, while several other species of *Bacillus* and *Staphylococcus* were identified at lower frequencies (5%). Overall, thirteen different species were identified by PCR, and *Escherichia coli* (64%), *Enterococcus* spp. (23%), *Trueperella pyogenes* and/or *Peptoniphilus indolicus* (18%), and *Staphylococcus* spp. (not including *S. aureus*; 14%) were the most common across flocks at early lactation. At peak lactation, *E. coli* (44%), and *Staphylococcus* spp. (32%) were still common as were *Klebsiella oxytoca* and/or *K. pneumoniae* (32%). The efficacy of SCC thresholds to predict culture status were assessed by receiver operating characteristic curves and Youden's Index (YI). Within MSU samples, YI was maximized at 240 x 10^3 and 480 x 10^3 cells/mL at early and peak lactation, respectively. Within USSES samples, YI was maximized at 1370 x 10^3 and 1050 x 10^3 cells/mL at early and peak lactation, respectively. The ideal thresholds to detect major pathogens (frequencies > 5%) across lactation stages for MSU and USSES samples were 1700 x 10^3 and 500 x 10^3 cells/mL, respectively. For minor pathogens (frequencies ≤ 5%), these thresholds were 250 x 10^3 and 760 x 10^3 cells/mL for MSU and USSES samples, respectively. Overall, the frequencies of bacteriologically milk samples indicate that subclinical mastitis is common

and prevalent in these flocks, and SCC thresholds can be used to infer intramammary infection in non-dairy ewes.

Key words: etiology, intramammary infection, mastitis, sheep, somatic cell count

INTRODUCTION

Economic losses contributing to mastitis in sheep can be substantial and include veterinary treatment, ewe culling and replacement, and reduced lamb performance (Timms, 2007). It has been estimated that, at a 10% incidence rate, mastitis costs the European Union (EU) dairy sheep and goat industry $67.4 million per year (Rupp and Foucras, 2010). In meat-type sheep, Conington et al. (2008) estimated that mastitis costs the UK purebred Texel industry over $3.5 million annually. Similar economic reports have not been conducted in the U.S. sheep industry, but udder health related issues, including hard-bag syndrome and mastitis, account for nearly 14% of ewes culled each year (USDA APHIS, 2011). Clinical symptoms of mastitis include swollen, firm, and feverish udders and abnormal milk (Menzies and Ramanoon, 2001; Gelasakis et al., 2015). However, subclinical mastitis (SCM) presents no observable symptoms in the milk or infected animal (Kahn et al., 2010). Still, ewes with SCM have an intramammary infection (IMI) detectable through bacterial screening of milk. These methods are often cost-prohibitive in many production settings, and somatic cell count (SCC) is commonly used to infer IMI status (Raynal-Ljutovac et al., 2007; Fragkou et al., 2014).

In dairy ewes, proposed SCC thresholds used to diagnose SCM range from 300 x 10^3 to over 2000 x 10^3 cells/mL (González-Rodríguez et al., 1995; Leitner et al., 2004). Though many bacterial genera have been implicated as causative pathogens for SCM, the most commonly isolated from dairy ewe cases include both coagulase positive and negative *Staphylococcus*, *Streptococcus*, *Bacillus*, *Trueperella*, *Escherichia*, *Mannheimia*, and *Pasteurella* globally (Gelasakis et al., 2015). The prevalence and etiology of SCM and utility of SCC to diagnose it has not been as extensively reported for non-dairy sheep. Therefore, the objectives of this study were to identify bacterial species present in milk and evaluate SCC thresholds relating to IMI in clinically healthy ewes managed in extensive production systems.

MATERIALS AND METHODS

All experimental protocols were approved by the Montana State University Agricultural Animal Care and Use Committee and the Institutional Animal Care and Use Committee of the U.S. Sheep Experiment Station.

Animal Management

Ewes in the present study were located at Montana State University's Red Bluff Research Ranch (MSU; Norris, MT) and the U.S. Sheep Experiment Station (USSES; Dubois, ID). MSU ewes were first bred to lamb at 2 yr of age. The ewes at MSU lambed in drylot during April and May and were moved indoors to single-ewe bonding pens for 12-36 h within 1 h of parturition. The ewe and lamb(s) were then transitioned through incrementally larger groups in single or twin mixing pens over the next week. During the

next 30-45 d, ewes grazed larger paddocks then grazed native range until weaning. MSU ewes were fed chopped grass (brome, garrison, and orchard) and alfalfa hay while in drylot and prior to summer grazing. Rangeland vegetation at MSU primarily consisted of bluebunch wheatgrass (*Pseudoroegneria spicata*), Idaho fescue (*Festuca idahoensis*), rubber rabbitbrush (*Ericameria nauseosa*), prairie sagewort (*Artemisia frigida*), lupine (*Lupinus* spp.), milkvetch (*Astragalus* spp.), and western yarrow (*Achillea millefolium*; Harris et al., 1989).

The ewes at USSES were managed similarly to those at MSU in that ewes were managed in drylot prior to lambing, moved indoors prior to lambing and bonded with their lamb(s) in individual pens, and transitioned through larger pens. However, USSES ewes were bred to lamb for the first time at 1 yr of age (rather than 2), lambed during March and April, and were placed in individual pens for a longer period (48-96 h). Furthermore, USSES ewes remained in drylot where they were fed a total mixed ration (45% alfalfa hay, 20% whole corn, 20% sugar beet pulp, 10% barley straw hay, 5% sugar beet condensed separator byproduct) with an added coccidiostat until summer grazing. At 30-45 d postpartum, USSES ewes and lambs were allocated to 1 of 2 bands and grazed sagebrush steppe until weaning. Typical rangeland vegetation at USSES consists primarily of mountain big sage brush, Sandberg bluegrass (*Poa secunda*), bluebunch wheatgrass (*Pseudoroegneria spicata*), sedge (*Carex* L.), and Idaho fescue (*Festuca idahoensis*). Additionally, dominant forbs include parsnip-flowered buckwheat (*Eriogonum heracleoides*), northwestern Indian paintbrush (*Castilleja angustifolia*), longleaf fleabane (*Erigeron corymbosus*), and littleleaf pussytoes (*Antennaria*

microphylla; Moffet et al., 2015). Mixed forb and short grass meadow communities dominated upper elevation sites, including slender wheatgrass (*Elymus trachycaulus*), oniongrass (*Melica bulbosa*), mountain brome (*Bromus marginatus*), sticky geranium (*Geranium viscosissimum*), mountain knotweed (*Polygonum douglasii*), narrowleaf collomia (*Collomia linearis*), and short-beaked agoseris (*Agoseris glauca*; Leytem and Seefeldt, 2008).

Milk Sampling

Milk samples were collected from clinically healthy ewes during the springs of 2017 (MSU only) and 2018. Study ewes were collected twice per year, corresponding to early- (2-5 d postpartum) and mid-lactation (30 – 45 d postpartum). Milk samples from first to third parity (2-4 yr) Rambouillet and Targhee ewes were sampled at MSU in 2017 (n = 28) and 2018 (n = 25). Samples from USSES were 1 to 7 yr-old Polypay, Columbia, Suffolk, and composite-line (⅜ Suffolk, ⅜ Columbia, ¼ Texel) ewes (n = 47). Prior to each collection, ewes were separated from lambs for ~30 min and administered ½ mL of oxytocin (VetOne; MWI Animal Health; Boise, ID) intramuscularly. Udders and teats were disinfected with 99% isopropyl alcohol, then the first 2-3 streams of residual milk were discarded. Ewes were then manually milked and 5- and 35-mL samples were aseptically obtained for bacterial speciation and SCC analysis, respectively. Milk samples were collected from each half of MSU ewes, whereas USSES ewes' milk samples were composited equally between halves. In total, 185 and 83 samples were collected from MSU (2017 = 85; 2018 = 100) and USSES ewes, respectively.

Quantification of Milk SCC

Milk samples collected for SCC analysis were preserved with 8 mg Bronopol and 0.3 mg Natamycin (Microtabs II; D & F Control Systems, Inc.; Dublin, CA) and refrigerated until in-house testing. Within 72 h of collection, a LactiCyte HD (Page & Pedersen International, Ltd.; Hopkinton, MA) somatic cell counter was used to quantify SCC of samples in duplicate. The equipment used precise fluorescent optics and low magnification to analyze images of tagged somatic cells. Somatic cell count was quantified and averaged across 16 images within each duplicate, which were then averaged across duplicates.

Bacterial Culturing

Milk samples collected for bacterial analysis were frozen and stored at -25°C until testing. Frozen milk samples were slowly thawed, then 1 mL was pipetted into a microcentrifuge tube. Samples were then centrifuged at 5000 x *g* for 5 min to separate fat, supernatant liquid, and milk pellet. Fat and the supernatant liquid were discarded, and the remaining pellet was vortexed (3000 rpm for ~5 s). A 10 µL inoculating loop was used to streak the pellet onto compartmentalized plates (Thermo Fischer Scientific Inc.; Waltham, MA) containing microbiological growth media. MacConkey Agar (HiMedia Laboratories Pvt. Ltd.; Mumbai, India), Trypticase Soy Agar (TSA; Becton, Dickinson and Company; Sparks, MD), TSA-5% sheep blood agar (Hardy Diagnostics; Santa Maria, CA), and Sabouraud Dextrose Agar (SDA; Neogen Corporation; Lansing, MI) were used as growth media to culture bacteria and yeasts. Plates were then incubated at 37°C for 18-24 h. Samples with growth were observed for number of colonies and

general morphology, and cultures that exhibited no growth were re-incubated for an additional 24 h. Culture-positive samples were further subcultured and used for identification via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

MALDI-TOF-MS Methods

Matrix-assisted laser desorption/ionization time-of-flight is a method of mass spectrometry to identify the source of an analyte. It utilizes a matrix to facilitate the ionization process of the analyte and separates groups of ions by mass to charge ratio (Alatoom et al., 2011; Saffert et al., 2011). The direct colony transfer method was used by transferring purified colonies in triplicate with a sterile 1-µL inoculating loop onto a 48-well steel-target plate. Then, 1 µL of Matrix A CHCA (alpha-cyano-4-hycroxycinnamic acid) was added to the center of each well. The procedures were conducted at the University of Wyoming (Laramie, WY) using an Agena MassArray Instrument (Agena Bioscience, San Diego, CA). If no replicate resulted in a successful match, samples were reanalyzed in triplicate.

Multiplex-PCR Methods

The Thermo Scientific PathoProof Complete-16 kit (Waltham, MA) was utilized according to the manufacturer's instructions with the PathoProof Complete-16 kit Primer Mix 4 for Applied Biosystems 7500 and 7500 Fast instruments. This test kit identifies 16 possible taxa commonly isolated from cases of bovine IMI, including: *Corynebacterium bovis*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella oxytoca* and/or *K. pneumoniae*, *Mycoplasma bovis*, *Mycoplasma* spp., *Prototheca* spp., *Serratia marcescens*,

Staphylococcal β-lactamase gene, *Staphylococcus aureus*, *Staphylococcus* spp., *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Trueperella pyogenes* and/or *Peptoniphilus indolicus*, and yeasts. Total DNA was extracted from frozen milk samples after similar preparation methods as culturing, then 4 PCR reactions were run for each milk sample (Eurofins DQCI, LLC.; Mounds View, MN).

Data Analyses

Results from bacterial speciation of MSU samples were first considered on an individual half basis, whereas USSES samples were analyzed on a whole udder basis. Within MSU ewes, bilateral negative, unilateral positive, and bilateral positive cases were calculated for culture and PCR methods. Persistence of infection or new infection across lactation was calculated for MSU and USSES flocks. The prevalence of culture- and PCR-positive samples within and across lactation stage were calculated within year (MSU only) and flock. When such calculations were compared across flocks, MSU results were considered on a whole-udder basis to coincide with composite samples from USSES. Here, unilateral and bilateral positive cases were both classified as "positive" on a whole-udder basis for MSU. Binomial proportions and exact (*i.e.*, Clopper-Pearson) 95% confidence intervals of taxa identified within culture- or PCR-positive samples were estimated using the *binom* package of R (R Core Team, 2019; Dorai-Raj, 2014).

Receiver operating characteristic (ROC) curves were constructed using the *ggplot2* (Wickham, 2016) and *plotROC* (Sachs, 2017) packages of R. These curves were used to assess optimal SCC thresholds that predict ewe culture status across lactation for each flock separately. To assess the accuracy of predicting ewe culture status from SCC,

sensitivity (Sen), specificity (Spe), positive predictive value (PPV), and negative predictive value (NPV) were also calculated at SCC thresholds corresponding to maximum Youden's Index (YI = Sen + Spe - 100; Youden, 1950).

RESULTS

Frequency of IMI and Taxa – MALDI-TOF-MS

In 2017, 24 and 18 MSU ewes had culture results from both halves within early and peak lactation, respectively, and 15 had culture results from both halves across lactation time points. The percentage of bilateral culture-negative, unilateral culture-positive, and bilateral culture-positive cases were 41.7, 54.2, and 4.2% in early lactation and 77.8, 22.2, and 0% in peak lactation, respectively. All MSU ewes sampled in 2018 had culture results from both halves at early and peak lactation. Again, in both early and peak lactation, cases of bilateral culture-negative (48 and 56%, respectively) and unilateral culture-positive (36 and 40%, respectively) were more common than bilateral culture-positive cases (16 and 4%, respectively). Within ewes sampled across lactation, most that were bilaterally culture-negative in early lactation remained so in peak lactation (2017 = 75%; 2018 = 66.7%). Of the ewes that had unilateral or bilateral culture-positive results in early lactation, 90.9% converted to bilateral culture-negative at peak lactation in 2017. However, most of the MSU ewes sampled in 2018 (53.8%) that were culture-positive in early lactation were culture-positive in peak lactation. Composite udder half milk samples were collected from USSES ewes and the majority (76.7%) had culture results at both early and peak lactation. Of the USSES ewes sampled twice, 27.8% were

culture-negative in early lactation and 60% of them remained so at peak lactation. Most of the USSES (57.7%) that were culture-positive in early lactation remained infected in peak lactation.

In total, 60 taxa were identified, and those at an overall frequency > 5% within positive samples are displayed (Tables 1 a & b). At early lactation, 63.4% of all samples on a whole-udder basis were culture-positive, with ranges from 30.6% (MSU ‘17, udder-half basis) to 74.4% (USSES, whole-udder basis; Table 1a). Across flocks in early lactation, 25% of culture-positive samples on a whole-udder basis had no taxa identified. The most commonly cultured bacteria within early lactation across flocks were *Bacillus altitudinis*, *B. amyloliquefaciens*, *B. licheniformis*, *B. subtilis*, *Staphylococcus auricularis*, *S. epidermidis*, and *Enterococcus faecium*. Of these, only *B. altitudinis*, *B. amyloliquefacien*, and *B. licheniformis* were cultured in both flocks and years at early lactation. Within the MSU flock at early lactation in 2017, *B. altidudinis* and *B. licheniformis* were the most prevalent species on an udder-half basis. During 2018, MSU cultures most often contained *B. altidudinis*, *B. amyloliquefaciens*, *B. licheniformis*, and *B. subtilis*. Within the USSES flock, *B. licheniformis* and *S. epidermidis* were the most common species in early lactation on a whole-udder basis.

At peak lactation, 42.9% of samples on a whole-udder basis were culture-positive across flocks. Whole-udder USSES samples had a greater incidence than udder-half MSU samples (Table 1b). Across flocks, the MALDI-TOF-MS methods were unable to identify 31% of culture-positive samples in peak lactation. The most commonly identified species across flocks were *B. altidudinis*, *B. licheniformis*, and other *Bacillus* spp., other

CoNS, and *S. aureus, S. auricularis, S. lugdunensis,* and *S. warneri*. During peak lactation at MSU in 2017, 75% of culture-positive udder-half samples were unidentified. The most commonly identified species within peak lactation at MSU in 2018 were *B. altitudinis* and other CoNS. Within peak lactation at USSES, *B. licheniformis* and other *Bacillus* spp. and *S. aureus, S. auricularis, S. lugdunensis,* and *S. warneri* were most prevalent on a whole-udder basis.

Frequency of IMI and Taxa – Multiplex-PCR

In 2017, 21 and 15 MSU ewes had PCR results from both halves within early and peak lactation, respectively, and 12 had PCR results from both halves across lactation time points. Of the 2017 MSU ewes with PCR results from both halves, all were bilaterally PCR-negative. Of the 25 MSU ewes sampled in 2018, 23 and 24 had PCR results from both halves within early and peak lactation, respectively, and 23 had PCR results from both halves across lactation. In early and peak lactation in 2018, cases of bilateral PCR-negative (87 and 83.3%, respectively) were most common, while unilateral (8.7 and 16.7%, respectively) and bilateral PCR-positive cases (4.3 and 0%, respectively) were less frequent. Within MSU ewes sampled across lactation in 2018, 95% of those that were bilaterally PCR-negative in early lactation remained so in peak lactation. Of the 3 ewes that had unilateral or bilateral PCR-positive results in early lactation, one converted to bilateral culture-negative at peak lactation. The majority of USSES ewes (70.2%) had PCR results at both early and peak lactation. Of the USSES ewes sampled twice, 51.5% were PCR-negative in early lactation and 41.2% of them remained so in

peak lactation. However, most of the USSES ewes (75%) that were PCR-positive in early lactation were also in peak lactation.

Overall, 13 taxa were identified by Multiplex-PCR within the test screen, and fewer samples were positive than what was observed for culture/MALDI-TOF-MS. Across flocks in early lactation, 25% of samples on a whole-udder basis were PCR-positive (Table 2a), and the most prevalent taxa were *Enterococcus* spp., *Escherichia coli*, *Staphylococcus* spp. (other than *S. aureus*), *Trueperella pyogenes* and/or *Peptinophilus indolicus*, and yeasts. No 2017 MSU udder-half samples were positive for the PCR test screen in early lactation. Within the MSU flock during 2018 at early lactation, 8.5% of samples were PCR-positive, and the most commonly isolated were *E. coli*, *Staphylococcus* spp., *T. pyogenes* and/or *P. indolicus*, and yeasts. In early lactation at USSES, 47.5% of whole-udder samples were PCR-positive, and *Enterococcus* spp., *E. coli*, and *T. pyogenes* spp. and/or *P. indolicus* were most common.

More whole-udder samples were PCR-positive (30.1%) in peak lactation compared to early lactation (Table 2b). Within the test screen, all of those identified in early lactation along with *K. oxytoca* and/or *K. pneumoniae*, Staphylococcal β-lactamase gene, and *S. aureus* were the most frequently isolated. Only one udder-half sample was PCR-positive in peak lactation at MSU in 2017 (yeasts). The taxa most commonly identified by PCR in peak lactation for 2018 MSU samples were *E. coli*, *S. aureus*, *Staphylococcus* spp., and Staphylococcal β-lactamase gene. Within USSES ewes at peak lactation, the most commonly isolated taxa on a whole-udder basis were *Enterococcus*

spp., *E. coli*, *K. oxytoca* and/or *K. pneumoniae*, *Staphylococcus* spp., and *T. pyogenes* and/or *P. indolicus*.

SCC Thresholds to Infer IMI

Due to the relatively low frequency of PCR-positive results for MSU samples, SCC thresholds were assessed within each flock using culture/MALDI-TOF-MS results only. In the context of the present study, sensitivity measures the proportion of culture-positive samples that were predicted to be positive based upon their SCC for a given threshold. Specificity is the proportion of culture-negative samples that were predicted to be negative based upon their SCC for a given threshold. Therefore, a perfect diagnostic test would have 100% Sen and Spe. Since this is not likely in experimental data, YI attempts to optimize Sen and Spe.

The ROC curves for MSU and USSES cultures across lactation stage and year (MSU only) are displayed in Fig. 1 a and b, respectively. In each curve, there is a distinct stair-step pattern which stays relatively close to the diagonal reference line. For MSU and USSES samples, YI was maximized at a SCC of 250 x 10^3 and 1050 x 10^3 cells/mL, respectively. Sensitivity, Spe, PPV, and NPV values at the corresponding maximum YI for total, major, and minor pathogens identified by MALDI-TOF-MS across lactation are displayed in Tables 3 a and b for MSU and USSES samples, respectively. Major pathogens for each flock were identified as taxa with frequencies > 5% across lactation stage, and minor pathogens were those with frequencies ≤ 5%. For MSU and USSES samples, YI were maximized at SCC thresholds of 1700 x 10^3 and 500 x 10^3 cells/mL, respectively, to identify the presence of major pathogens. When considering minor

pathogens in MSU and USSES samples, YI were maximized at 250 x 10^3 and 760 x 10^3 cells/mL, respectively.

DISCUSSION

Frequency of IMI

In the present study, the frequency of IMI across flocks and years were generally greater than past research has reported. Additionally, there was a much lower frequency of PCR-positive samples compared to culture-positive samples at both early and peak lactation. A likely explanation for this is the limited number of taxa included in the PCR test kit's screen (n = 16). In reviewed literature, the greatest frequency of IMI in non-dairy ewes was calculated to be 30% across 22 flocks in Sweden (Persson et al., 2017). In other European countries, frequencies ranged from 12% across 7 flocks in England (Watkins et al., 1991) to 17% in Finland (Maisi et al., 1987). Similarly, Watson et al. (1990) reported a 14% incidence across 8 Australian flocks. Across 30 Canadian flocks, Arsenault et al. (2008) determined the frequency of IMI to be 18%. Most studies investigating IMI in U.S. sheep populations have been limited to a single flock. Ahmad et al. (1992a) reported an IMI frequency of 24.5% at partition and 21.9% at weaning in a flock of crossbred ewes in Iowa. Keisler et al. (1992) sampled ewes multiple times from 0 to 8 wk postpartum and reported IMI frequencies ranging from 11 to 27%. Within flock and lactation stage frequencies of IMI in the present study ranged from 11 to 74%. The present research is unique in that extensively managed ewes were sampled across 2 flocks and 2 lactation stages. Results show flock and lactation stage dependencies on

frequency of IMI, which may be attributed to different pathogens or environments. Therefore, results from the present and past studies provide evidence that of the proportion of ewes with IMI is largely variable across lactation stages and flocks but is generally common.

Frequency of Taxa

Watkins et al. (1991) and Clements et al. (2003) both reported that *Mannheimia* spp. were among the most commonly isolated species from cases of SCM in nursing ewes, at frequencies of 23 and 10%, respectively. However, other researchers have shown lower frequencies of *Mannheimia* spp. (<5%; Hariharan et al., 2004; Arsenault et al., 2008). In the present study, *Mannheimia* spp. were not identified by culture and MALDI-TOF-MS and were not included in the PCR screen. Furthermore, *Staphylococcus aureus* was another commonly isolated bacterium in cases of SCM in meat-type ewes in reviewed literature, with frequencies as high as 31 to 40% (Watson et al., 1990; Arsenault et al., 2008) in subclinically infected ewes and between 45 and 65% of clinical cases (Mørk et al., 2007; Koop et al., 2010). In the present study, *S. aureus* was identified at a low frequency (≤ 5%) using both culture and PCR methods.

Streptococcus spp. have also been isolated from 5% of clinically infected ewes (Mørk et al., 2007; Blagitz et al., 2014) and 6-42% in ewes with SCM (Watkins et al., 1991; Arsenault et al., 2008). Of the *Streptococcus* spp. isolated, *S. aglactiae*, *S. uberis*, and *S. bovis* are reported to be common (Ariznabarreta et al., 2002), but MALDI-TOF-MS only identified *S. mitis* and *S. suis* at low levels in the present study (≤ 2%). Still, Watkins et al. (1991) and Arsenault et al. (2008) reported that *Bacillus* spp. are prevalent

(2.1-20.8%) in subclinically infected non-dairy ewes, which agrees with the present study. In the reviewed literature, *Escherichia coli* was the most common species of its genus isolated in ewe milk (Watkins et al., 1991; Lafi et al., 1998; Mørk et al., 2007), and was the most commonly identified taxa by PCR across flocks and years in the present study.

Therefore, large variation exists in the identities of bacteria species isolated in milk samples collected from clinically healthy ewes. In the present study, more than 60 bacteria taxa were identified using MALDI-TOF-MS. This large number could be attributed to different environmental bacteria, since ewes and lambs were transitioned from close confinement to drylot and pasture throughout lactation in both flocks.

SCC Thresholds to Infer IMI

The statistics estimated in the present study have all been previously utilized to analyze the efficacy of predicting IMI status from SCC. A majority of the research has been conducted in dairy cows, which generally agree on SCC thresholds from 100 x 10^3 to 400 x 10^3 cells/mL (Dohoo et al., 1981; Dohoo and Leslie, 1991; Malek dos Reis et al., 2011). However, past research in dairy ewes has not agreed on such a narrow range of thresholds to infer IMI status. González-Rodríguez et al. (1995) determined that 300 x 10^3 cells/mL was the optimal threshold in Spanish dairy ewes with sensitivity and specificity values of 79.5 and 82.2%, respectively. In Valle del Belice ewes, Riggio et al. (2013) estimated an optimal threshold at 645 x 10^3 cells/mL, where Sen, Spe, PPV, and NPV values were 53, 86, 69, and 76%, respectively. Suarez et al. (2002) estimated mean SCC of healthy and subclinically infected dairy ewes of 375 x 10^3 and 1464 x 10^3

cells/mL, respectively. Furthermore, at a threshold of 1200 x 10^3 cells/mL, Sen, Spe, PPV, and PPV were 73, 87, 78, and 83%, respectively.

Fewer studies have investigated SCC thresholds that indicate IMI in non-dairy ewes. Clements et al. (2003) reported that Sen and Spe were equal (67%) at a threshold of 1284 x 10^3 cells/mL. Similarly, Maisi et al. (1987) determined a threshold of 1660 x 10^3 cells/mL was optimal and resulted in Spe and Sen values of 82% in Finnsheep, Texel, and their crossbreds. However, Świderek et al. (2016) estimated relatively high Sen (74%) and Spe (66%) at a much lower SCC threshold (205 x 10^3 cells/mL) in Polish Heath and Lowland ewes. Fthenakis et al. (1991) evaluated SCC thresholds from 500 x 10^3 to 1500 x 10^3 cells/mL to determine the optimal threshold to determine subclinical mastitis. This threshold corresponded to 1000 x 10^3 cells/mL, which resulted in Spe and Sen values of 98 and 86%, respectively. In the present study, YI was maximized between 240 x 10^3 and 1370 x 10^3 cells/mL using culture methods within MSU and USSES flocks. The SCC which maximized YI also varied by lactation stage and pathogen classification. Still, these thresholds are within the large range of those suggested by previous researchers for non-dairy sheep and no literature was found to suggest a SCC threshold in extensively managed meat- or fine-wool- type ewes in the Western U.S. Potential explanations for variation in literature estimates of SCC thresholds may include breed differences in immune response, management system (intensive vs. extensive), and causative pathogens (González-Rodríguez et al., 1995; Suarez et al., 2002).

Conclusions

The etiology of ovine subclinical mastitis in two research flocks in the Western U.S. reported here indicate a variety of potentially pathogenic bacteria are commonly isolated in milk collected from otherwise healthy ewes. The frequency of intramammary infection was generally high, but variable across flocks, years, and lactation stages. Commonly isolated bacteria in the reviewed literature were of the *Staphylococcus*, *Streptococcus*, *Bacillus*, *Mannheimia*, and *Escherichia* genera. In the present study, no *Mannheimia* spp. were isolated and the frequencies of *Streptococcus* spp. were low. Nevertheless, *E. coli*, *Staphylococcus* spp., and *Bacillus* species were common. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was one method used in the present study to classify bacterial growth on culture. This newer technology was not a common method in past research investigating ovine intramammary infection. Additionally, environment and management systems and practices are likely to attribute to some variation in bacteria isolated and identified from milk samples. In dairy cows and ewes, somatic cell count thresholds to infer intramammary infection have been well researched, yet substantially fewer studies have evaluated non-dairy ewes. The results from the present study indicate milk somatic cell count can be used to infer intramammary infection in extensively managed ewes, but thresholds vary across flocks and lactation stage. In conclusion, all forms of mastitis are common in meat-type ewes and emphasis should be placed on identifying cost-effective husbandry practices to mitigate its effects.

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Table 1a. Number of samples collected in early lactation within and across flocks and estimated binomial proportions with associated 95% confidence intervals of most commonly isolated species (Total > 0.05) within culture-positive samples using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) methods.

	Flock (year)[1]			
Item	MSU (‘17)	MSU (‘18)	USSES	Total
No. samples[2]	49	50	43	93
No. positive (%)[3]	15 (30.6)	17 (34.0)	32 (74.4)	59 (63.4)
Unidentified[4]	0.27 [0.08, 0.55]	0.24 [0.07, 0.50]	0.22 [0.09, 0.40]	0.25 [0.15, 0.38]
B. altitudinis	0.27 [0.08, 0.55]	0.18 [0.04, 0.43]	0.03 [0.00, 0.16]	0.12 [0.05, 0.23]
B. amyloliquefaciens	0.07 [0.00, 0.32]	0.18 [0.04, 0.43]	0.06 [0.01, 0.21]	0.10 [0.04, 0.21]
B. licheniformis	0.13 [0.02, 0.40]	0.12 [0.01, 0.36]	0.25 [0.11, 0.43]	0.20 [0.11, 0.33]
B. subtilis	0.00 [0.00, 0.22]	0.18 [0.04, 0.43]	0.06 [0.01, 0.21]	0.08 [0.03, 0.19]
Ent. faecium	0.00 [0.00, 0.22]	0.00 [0.00, 0.20]	0.12 [0.04, 0.29]	0.07 [0.02, 0.16]
S. auricularis	0.00 [0.00, 0.22]	0.00 [0.00, 0.20]	0.12 [0.04, 0.29]	0.07 [0.02, 0.16]
S. epidermidis	0.00 [0.00, 0.22]	0.06 [0.00, 0.29]	0.22 [0.09, 0.40]	0.14 [0.06, 0.25]

[1]MSU = Montana State University; USSES = U.S. Sheep Experiment Station.
[2]MSU ‘17 and ‘18 samples considered on an udder-half basis, USSES and Total samples considered on a whole-udder basis.
[3]Number and percent of samples that exhibited growth on culture and were speciated by MALDI-TOF-MS, species proportions are within culture positive samples.
[4]Unidentified = samples that were culture positive but not identified by MALDI-TOF-MS; *B.* = *Bacillus*; *Ent.* = *Enterococcus*; *S.* = *Staphylococcus*.

Table 1b. Number of samples collected in peak lactation within and across flocks and estimated binomial proportions with associated 95% confidence intervals of most commonly isolated species (Total > 0.05) within culture-positive samples using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) methods.

	Flock (year)[1]			
Item	MSU (‘17)	MSU (‘18)	USSES	Total
No. samples[2]	37	50	40	84
No. positive (%)[3]	4 (10.8)	12 (24.0)	21 (52.5)	36 (42.9)
Unidentified[4]	0.75 [0.19, 0.99]	0.33 [0.10, 0.65]	0.19 [0.05, 0.42]	0.31 [0.16, 0.48]
B. altitudinis	0.00 [0.00, 0.60]	0.08 [0.00, 0.38]	0.05 [0.00, 0.24]	0.06 [0.01, 0.19]
B. licheniformis	0.00 [0.00, 0.60]	0.00 [0.00, 0.26]	0.24 [0.08, 0.47]	0.14 [0.05, 0.29]
Other *B.* spp.	0.00 [0.00, 0.60]	0.00 [0.00, 0.26]	0.10 [0.01, 0.30]	0.06 [0.01, 0.19]
S. aureus	0.00 [0.00, 0.60]	0.00 [0.00, 0.26]	0.10 [0.01, 0.30]	0.06 [0.01, 0.19]
S. auricularis	0.00 [0.00, 0.60]	0.00 [0.00, 0.26]	0.10 [0.01, 0.30]	0.06 [0.01, 0.19]
S. lugdunensis	0.00 [0.00, 0.60]	0.00 [0.00, 0.26]	0.10 [0.01, 0.30]	0.06 [0.01, 0.19]
S. warneri	0.00 [0.00, 0.60]	0.00 [0.00, 0.26]	0.10 [0.01, 0.30]	0.06 [0.01, 0.19]
Other CoNS	0.00 [0.00, 0.60]	0.08[0.00, 0.38]	0.05 [0.00, 0.24]	0.06 [0.01, 0.19]

[1]MSU = Montana State University; USSES = U.S. Sheep Experiment Station.
[2]MSU ‘17 and ‘18 samples considered on an udder-half basis, USSES and Total samples considered on a whole-udder basis.
[3]Number and percent of samples that exhibited growth on culture and were speciated by MALDI-TOF-MS, species proportions are within culture positive samples.
[4]Unidentified = samples that were culture positive but not identified by MALDI-TOF-MS; *B.* = *Bacillus*; *S.* = *Staphylococcus*; CoNS = Coagulase negative staphylococci.

Table 2a. Number of samples collected in early lactation within and across flocks and estimated binomial proportions with associated 95% confidence intervals of most commonly isolated species (Total > 0.05) within positive samples using Multiplex-PCR methods.

	Flock (year)[1]			
Item	MSU (‘17)	MSU (‘18)	USSES	Total
No. samples[2]	45	47	40	88
No. positive (%)[3]	0	4 (8.5)	19 (47.5)	22 (25.0)
Ent. spp.[4]	-	0.00 [0.00, 0.60]	0.26 [0.09, 0.51]	0.23 [0.08, 0.45]
E. coli	-	0.25 [0.01, 0.81]	0.68 [0.43, 0.87]	0.64 [0.41, 0.83]
T. pyogenes and/or *P. indolicus*	-	0.25 [0.01, 0.81]	0.16 [0.03, 0.40]	0.18 [0.05, 0.40]
S. spp.	-	0.50 [0.07, 0.93]	0.05 [0.00, 0.26]	0.14 [0.03, 0.35]
Yeasts	-	0.25 [0.01, 0.81]	0.05 [0.00, 0.26]	0.09 [0.01, 0.29]

[1]MSU = Montana State University; USSES = U.S. Sheep Experiment Station.
[2]MSU ‘17 and ‘18 samples considered on an udder-half basis, USSES and Total samples considered on a whole-udder basis.
[3]Number and percent of samples that exhibited positive Multiplex-PCR results, species proportions are within positive samples.
[4]*Ent. = Enterococcus*; *E. = Escherichia*; *K. = Klebsiella*; *T. = Trueperella*; *P. = Peptoniphilus*; *S. = Staphylococcus*.

Table 2b. Number of samples collected in peak lactation within and across flocks and estimated binomial proportions with associated 95% confidence intervals of most commonly isolated species (Total > 0.05) within positive samples using Multiplex-PCR methods.

	Flock (year)[1]			
Item	MSU '17	MSU '18	USSES	Total
No. samples[2]	33	49	39	83
No. positive (%)[3]	1 (3.0)	5 (10.2)	19 (48.7)	25 (30.1)
Ent. spp.[4]	0	0.00 [0.00, 0.52]	0.11 [0.01, 0.33]	0.08 [0.01, 0.26]
E. coli	0	0.60 [0.15, 0.95]	0.42 [0.20, 0.67]	0.44 [0.24, 0.65]
K. oxytoca and/or *K. pneumoniae*	0	0.00 [0.00, 0.52]	0.42 [0.20, 0.67]	0.32 [0.15, 0.54]
T. pyogenes and/or *Peptoniphilus indolicus*	0	0.00 [0.00, 0.52]	0.11 [0.01, 0.33]	0.08 [0.01, 0.26]
Staphylococcal β-lactamase gene	0	0.20 [0.01, 0.72]	0.05 [0.00, 0.26]	0.08 [0.01, 0.26]
S. aureus	0	0.20 [0.01, 0.72]	0.05 [0.00, 0.26]	0.08 [0.01, 0.26]
S. spp.	0	0.60 [0.15, 0.95]	0.26 [0.09, 0.51]	0.32 [0.15, 0.54]

[1]MSU = Montana State University; USSES = U.S. Sheep Experiment Station.
[2]MSU '17 and '18 samples considered on an udder-half basis, USSES and Total samples considered on a whole-udder basis.
[3]Number and percent of samples that exhibited positive Multiplex-PCR results, species proportions are within positive samples.
[4]*Ent. = Enterococcus*; *E. = Escherichia*; *K. = Klebsiella*; *T. = Trueperella*; *P. = Peptoniphilus*; *S. = Staphylococcus*.

Table 3a. Somatic cell count threshold corresponding to the maximum Youden's Index (SCC_{YImax}) and associated sensitivity (Sen), specificity (Spe), positive predictive value (PPV), and negative predictive value (NPV) statistics for total, major, and minor pathogens identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry across lactation stage and year for Montana State University samples.

Pathogen grouping[1]	SCC_{YImax}, cells/mL	Statistic, %			
		Sen	Spe	PPV	NPV
Total	250 x 10^3	70.8	39.1	28.8	79.4
Major	1700 x 10^3	11.8	90.5	11.1	91.1
Minor	250 x 10^3	69.6	37.4	13.6	89.7

[1]Total = including all identified and unidentified taxa.
Major = taxa identified in culture-positive samples at frequencies > 5% including *Bacillus altitudinis*, *B. amyloliquefaciens*, *B. licheniformis*, and *B. subtilis*.
Minor = taxa identified in culture-positive samples with frequencies ≤ 5% including *Actinobaculum schaalii*; *Bacillus firmus*, *B. groupe cereus*, and *B. pumilus*; *Bacteroides fragilis*; *Corynebacterium mastitidis* and *C. simulans*; *Haemophilus parainfluenzae*; *Heliobacter pullorum*; *Micrococcus flavus* and *M. luteus*; *Paenibacillus campinasensis*; *Sphingomonas paucimobilis*; *Staphylococcus cohnii*, *S. epidermidis*, *S. hominis*, *S. intermedius*, *S. lugdunensis*, *S. pseudintermedius*, and *S. succinus*; other coagulase-negative Staphylococci; other *Staphylococcus* spp.; *Streptococcus mitis*, *Strep.* groupe mitis, and *Strep.* groupe sanguinis.

Table 3b. Somatic cell count threshold corresponding to the maximum Youden's Index (SCC_{YImax}) and associated sensitivity (Sen), specificity (Spe), positive predictive value (PPV), and negative predictive value (NPV) statistics for total, major, and minor pathogens identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry across lactation stage for U.S. Sheep Experiment Station samples.

Pathogen grouping[1]	SCC_{YImax}, cells/mL	Statistic, %			
		Sen	Spe	PPV	NPV
Total	1050×10^3	45.3	46.7	68.6	42.5
Major	500×10^3	66.7	38.0	44.0	76.0
Minor	760×10^3	62.1	46.3	42.9	75.8

[1]Total = including all identified and unidentified taxa.
Major = taxa identified in culture-positive samples at frequencies > 5% including *Bacillus licheniformis* and other *Bacillus* spp.; *Enterococcus faecium*; and *Staphylococcus auricularis*, *S. epidermidis*, and *S. warneri*.
Minor = taxa identified in culture-positive samples with frequencies ≤ 5% including *Acinetobacter lwoffi*; *Acitinobaculum schaalii*; *Aerococcus viridians*; *Bacillus altitudinis*, *B. amyloliquefaciens*, and *B. subtilis*; *Candida lusitaniae*; Corynebacterium *amycolatum*, *C. appendicitis*, and other *Corynebacterium* spp.; *Escherichia coli* and *E. hirae*; *Haemophilus parainfluenzae*; *Heliobacter pylori*; *Kytococcus schroeteri*; *Microbacterium testaceum*; *Paenibacillus polymyxa*; *Rhodotorula mucilaginosa*; *Rothia mucilaginosa*; *Staphylococcus aureus*, *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*; other coagulase-negative Staphylococci; *Streptococcus suis*; and *Turicella otitidis.*

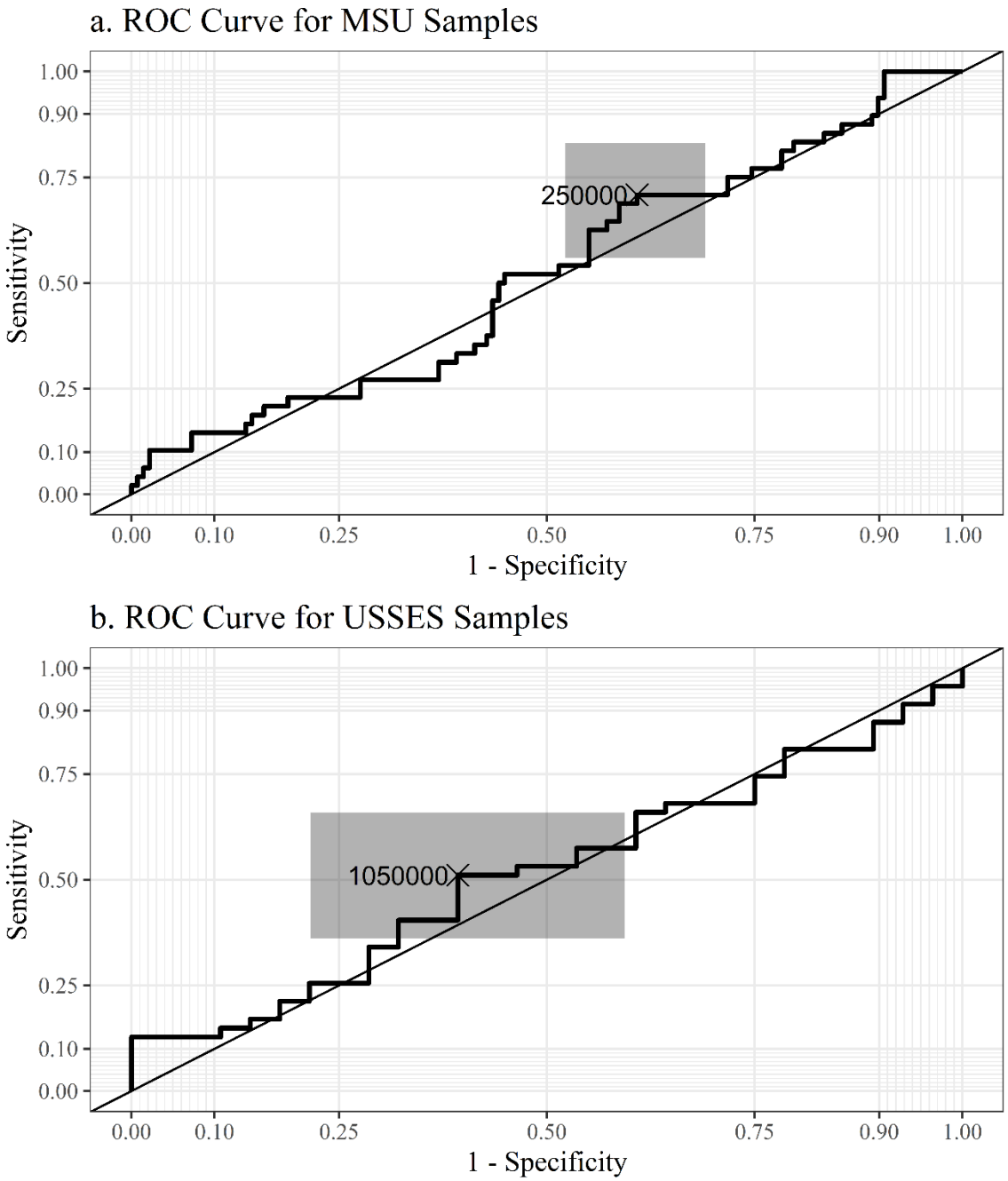

Figure 1. Receiver Operator Characteristic (ROC) curves to assess the ability of somatic cell count (SCC) thresholds to predict culture status of Montana State University (MSU; a) and U.S. Sheep Experiment Station (USSES; b) samples. The SCC threshold (cells/mL) corresponding to the maximum Youden's Index value and its 95% confidence interval are indicated.

CHAPTER FOUR

UDDER MORPHOMETRY AND HEALTH AND THEIR RELATIONSHIP WITH EWE SOMATIC CELL COUNT AND PRODUCTIVITY IN EXTENSIVELY MANGED RESEARCH FLOCKS

Contribution of Authors and Co-Authors

Manuscript in Chapter 4

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Contributions: Contributed in data collection, primary role in data analysis, interpretation of results, and manuscript preparation.

Co-Author: W. C. Stewart

Contributions: Co-principal investigator, assisted in study conception, obtaining funding, experimental design, data collection, and manuscript preparation.

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Udder morphometry and health and their relationship with ewe somatic cell count and productivity in extensively managed research flocks[1,2]

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ABSTRACT: Clinical mastitis is an important disease to all sheep producers from both an economic and animal welfare perspective. However, past research has indicated that subclinical mastitis is the more common form of the disease but is cost-prohibitive to diagnose at the production level. Therefore, the economic impact of subclinical mastitis and selection strategies to reduce its occurrence are not well understood in non-dairy sheep. The objectives of this study were to estimate the effect of somatic cell count (SCC) on ewe productivity and relationships among udder and teat morphometric traits and SCC in 2 Western U.S. research flocks (Montana State University, MSU; U.S. Sheep Experiment Station, USSES). Milk was collected from clinically healthy ewes during early (< 5 d) and peak lactation (30-45 d) and used to quantify SCC. Ewe productivity was defined as total 120 d adjusted litter weight (LW120) and analyzed within flock with class effects of ewe breed, parity, and year and the linear covariate of $\log_{10}$ SCC at early ($LSCC_E$) or peak lactation ($LSCC_P$). The effect of LSCC on ewe productivity was

dependent on lactation stage and production year, but when significant, indicated a 9.2-14.7 kg reduction in LW120 associated with a 1-unit increase in LSCC. Udder and teat characteristics were measured or scored on a linear scale at each sampling and included teat length (TL), udder symmetry (SYM), presence of supernumerary teats (SNT), and external damage to the teat (EDT), to name a few. A stepwise model selection approach was used to determine the most parsimonious models to predict LSCC. Main effects of parity, year, and SNT were included in the final model for USSES ewes but combined to explain only 27% of the variation in $LSCC_P$. Nevertheless, 1-yr old USSES ewes had 6% greater ($P < 0.01$) $LSCC_P$ than 3-yr and older ewes. The main effects of year, TL, EDT, SYM, and SNT explained 51% of the variation in $LSCC_E$ for MSU ewes. Ewes with TL greater than 3.3 cm had 7-8% greater ($P \leq 0.02$) $LSCC_E$ than ewes with TL less than 3 cm. Additionally, ewes with EDT or SNT had 4 and 8% less ($P < 0.01$) $LSCC_E$ than ewes without EDT or SNT, respectively. Results indicated that subclinical mastitis is common in extensively managed flocks and has major economic implications. However, associations between udder and teat morphometric traits and LSCC were inconsistent. Therefore, additional studies investigating effective methods of reducing mastitis in meat- and wool-type ewes are warranted.

Keywords: ewe productivity, mastitis, somatic cell count, udder health, udder morphometry

INTRODUCTION

Mastitis caused by bacterial infection is an economically devastating disease to all sheep producers. Ewes displaying symptoms such as swollen, firm, and feverish udders

experience clinical mastitis which is easier to diagnose at the production level than subclinical mastitis. In non-dairy sheep systems, production impacts of mastitis include ewe culling (USDA APHIS 2011), veterinary treatments, and reduced lamb survival (Holmøy et al., 2014) and growth (Gross et al., 1978; Fthenakis and Jones, 1990b; Ahmad et al., 1992a). While somatic cell count (SCC) is commonly used to infer subclinical mastitis, it is lowly heritable in both dairy (El-Saied et al., 1999; Hamann et al., 2004; Murphy et al., 2017b) and non-dairy ewes (Crump et al., 2018; McLaren et al., 2018). Furthermore, quantifying SCC in large, extensively managed flocks is not practical in a commercial setting. Linear scoring systems have been developed to evaluate ewes for various mammary traits such as udder depth and shape, and teat length and placement. Genetic parameters between udder morphometric traits, milk yield, and SCC have been estimated in dairy ewes and indicated selection may be effective (de la Fuente et al., 1996; Serrano et al., 2002; Casu et al., 2006). More recently, similar studies have investigated the relationship between udder morphometry, productivity, and subclinical mastitis in non-dairy ewes (Cooper et al., 2013; Crump et al., 2018). Such cost-effective and easily measured indicator traits could be a means of reducing mastitis in non-dairy ewes. For example, McLaren et al. (2018) estimated strong genetic correlations (0.61 to 0.75) between mastitis traits and udder depth in Texel ewes. The objectives of this study were to estimate relationships between udder and teat morphometric traits, SCC, and productivity in extensively managed, meat- and wool-type ewes.

MATERIALS AND METHODS

Animals and Management

Ewes considered in the present study were located at Montana State University's Red Bluff Research Ranch (MSU; Norris, MT) and the U.S. Sheep Experiment Station (USSES; Dubois, ID). MSU ewes were first bred to lamb at 2 yr of age in drylot during April and May. Within 1 h of parturition ewes were moved indoors to individual bonding pens for 12 to 36 h. The ewe and her lamb(s) were then transitioned through incrementally larger groups in single or twin mixing pens over the next 7 d. Ewes grazed larger paddocks for 30-45 d, then were herded as 1 contiguous band on native range until weaning. MSU ewes were fed chopped grass (brome, garrison, and orchard) and alfalfa hay while in drylot and prior to summer grazing. Rangeland vegetation at MSU primarily consisted of bluebunch wheatgrass (*Pseudoroegneria spicata*), Idaho fescue (*Festuca idahoensis*), rubber rabbitbrush (*Ericameria nauseosa*), prairie sagewort (*Artemisia frigida*), lupine (*Lupinus* spp.), milkvetch (*Astragalus* spp.), and western yarrow (*Achillea millefolium*; Harris et al., 1989).

Pre- and postpartum ewe management at USSES was similar to MSU in that ewes were managed in drylot prior to lambing and were bonded to their lamb(s) in individual pens before transitioning through larger mixing pens. However, USSES ewes were bred to lamb for the first time at 1 yr of age (rather than 2), lambed during March and April, and were placed in individual pens for a longer period (48 h up to 96 h). Additionally, USSES ewes remained in drylot until summer grazing where they were fed a total mixed ration consisting of alfalfa hay (45%), whole corn (20%), sugar beet pulp (20%), barley

straw hay (10%), and sugar beet condensed separator byproduct (5%) with an added coccidiostat. At 30-45 d postpartum, USSES ewes and lambs were allocated to 1 of 2 bands and grazed sagebrush steppe until weaning. Typical rangeland vegetation at USSES consists primarily of mountain big sage brush, Sandberg bluegrass (*Poa secunda*), bluebunch wheatgrass (*Pseudoroegneria spicata*), sedge (*Carex* L.), and Idaho fescue (*Festuca idahoensis*). Additionally, dominant forbs include parsnip-flowered buckwheat (*Eriogonum heracleoides*), northwestern Indian paintbrush (*Castilleja angustifolia*), longleaf fleabane (*Erigeron corymbosus*), and littleleaf pussytoes (*Antennaria microphylla*; Moffet et al., 2015). Upper elevation sites were dominated by mixed forb and short grass meadow communities, including slender wheatgrass (*Elymus trachycaulus*), oniongrass (*Melica bulbosa*), mountain brome (*Bromus marginatus*), sticky geranium (*Geranium viscosissimum*), mountain knotweed (*Polygonum douglasii*), narrowleaf collomia (*Collomia linearis*), and short-beaked agoseris (*Agoseris glauca*; Leytem and Seefeldt, 2008).

MSU Targhee and Rambouillet ewes (2- to 6-yr-old) included in the present study were randomly selected from the larger flock approximately 5 d after parturition in 2017 and 2018. Ewes at USSES had been previously allocated to experiments prior to sampling and inclusion in the present study. One- to 7-yr-old Suffolk and terminal composite (⅜ Suffolk, ⅜ Columbia, ¼ Texel) ewes were sampled solely to survey SCC and udder characteristics (Survey) in 2017 and 2018. Additionally, 1-yr-old USSES Rambouillet, Suffolk, and Targhee ewes were in the control treatment (Control) of a study that investigated the effects of maternal periparturient chlorate salt

supplementation. A component this study was to quantify the effect of treatment on milk SCC and ewes were sampled in 2017 and 2018.

Milk Collection and SCC Quantification

Milk was collected from all previously described MSU and USSES ewes, all of which were deemed clinically healthy at the time of sampling to be included in analyses of the present study. Prior to each collection, ewes were separated from their lamb(s) for ~30 min, administered ½ mL of oxytocin (VetOne; MWI Animal Health; Boise, ID) intramuscularly, and restrained on a milking stand. Teats were then disinfected, the first 2-3 streams of residual milk were discarded, and ewes were manually milked. Milk samples were collected on MSU and USSES Control ewes shortly after lambing (3-5 d; Early) and prior to turn-out to summer grazing (35-45 d; Peak). USSES Survey ewes were only collected at Peak lactation. Milk samples were collected separately from each udder half for MSU and USSES Survey ewes and composited equally between udder halves for USSES Control ewes. Approximately 35 mL milk samples were obtained for SCC quantification and preserved with 8 mg Bronopol and 0.3 mg Natamycin (Microtabs II; D & F Control Systems, Inc.; Dublin, CA) then refrigerated until in-house testing. Within 72 h of collection, a LactiCyte HD (Page & Pedersen International, Ltd.; Hopkinton, MA) was used to quantify SCC of samples in duplicate. This equipment used precise fluorescent optics and low magnification to analyze images of tagged somatic cells. The SCC is quantified and averaged across 16 images within each duplicate, which were then averaged across duplicates for each sample.

Ewe Udder, Teat, and Anatomical Traits

At the time of milk sampling, all MSU ewes and USSES Survey ewes had udder and teat morphometric traits collected (Table 1). Objective traits were determined by a ruler or tape measure and included: teat length (TL), udder length (UL), and udder circumference (UC). Subjective traits of udder morphometry were adapted from Casu et al. (2006) and included teat placement (TP) and degree of separation of udder halves (DS), both using a 9-point visual scale available to the grader at the time of sampling. A subjective scoring system was also developed for udder symmetry (SYM), udder palpation score (PALP) to detect intramammary masses, and degree of external damage to teats (EDT). Supernumerary teats (SNT) were defined as the presence/absence of more than 2 functional or non-functional teats. Udder wool score (UWS) quantified the degree of wool coverage over the entire mammary gland. Finally, body condition score (BCS) was also assessed at this time and expressed on a 1 (very thin) to 5 (obese) scale.

Ewe Productivity

Lambs born to all sampled ewes were weighed within 12 h of birth and at weaning (127 ± 6 d). Birth weight was used to linearly adjust lamb weaning weight to 120 d, which was then summed within ewe. Here, a ewe's total 120 d litter weight (LW120) evaluates maternal productivity and is a composite trait that combines the joint effects of lamb sex, survival, and growth to weaning. Therefore, if a ewe's lamb(s) was transferred to the nursery or died prior to weaning, its record did not contribute to LW120.

Statistical Analyses

Ewes with a SCC greater than 2000 x 10^3 cells/mL were identified as outliers and removed from their respective data set. This limited the chance of inadvertently including clinically infected ewes or inaccurate SCC readings. After data cleaning, records from a total of 74, 157, and 81 MSU, USSES Survey, and USSES Control ewes, respectively, were used in the following analyses. Somatic cell count was first transformed to the $\log_{10}$ scale (LSCC) for udder half and udder composite milk samples. The CORR procedure of SAS (v. 9.4; SAS Inst. Inc., Cary, NC) was used to estimate Pearson correlation coefficients between udder half LSCC within and across collection point for MSU and USSES Survey ewes separately. Udder half LSCC were then averaged within collection for MSU and USSES Survey ewes ($LSCC_C$). Correlation coefficients between $LSCC_C$ across lactation were estimated for MSU, USSES Survey, and USSES Control ewes separately.

To estimate factors contributing to ewe productivity, LW120 was analyzed in separate models for MSU, USSES Survey, and USSES Control ewes using the GLM procedure of SAS. Fixed classification effects included ewe parity (1, 2, or 3+), production year (2017 or 2018), and ewe breed (MSU = Rambouillet or Targhee; USSES Survey = Suffolk or terminal composite; USSES Control = Rambouillet, Suffolk, or Targhee). Additionally, the linear covariate of $LSCC_C$ at each collection point (nested within main effects) was fit separately and all 2-way interactions among classification effects were considered and subsequently removed if not significant ($P > 0.05$).

The GLMSELECT procedure was then used to evaluate models for the prediction of $LSCC_C$ in MSU and USSES Survey ewes. All measured traits from Table 1 were placed into classes and fit as main effects along with ewe parity, production year, breed, and number of lambs born (1 or 2+). Here, a stepwise approach was taken where the combination of main effects that resulted in the minimum Akaike Information Criterion was determined to be the most parsimonious model. Least-squares means of main effect levels and pairwise differences among them were then estimated by fitting the selected model in the GLM procedure.

RESULTS

Ewe SCC and Productivity

Within MSU ewes, moderate to strong positive correlations were estimated for LSCC between udder halves in both early (0.50) and peak lactation (0.92; Table 2). However, correlations between udder half LSCC and $LSCC_C$ across lactation ranged from non-significant (0.05-0.21; $P \geq 0.07$) to moderate (0.23-0.32; $P \leq 0.05$). Within USSES Survey ewes, the correlation between left and right udder half LSCC at peak lactation was moderate and positive (0.58), and both were strongly correlated with $LSCC_C$ (0.89-0.91; $P < 0.01$). The estimated correlation coefficient between early and peak lactation $LSCC_C$ was also moderate (0.35; $P < 0.001$) for USSES Control ewes.

Least-squares means for the main classification effects and solutions for the linear covariate of $LSCC_C$ on LW120 for MSU and USSES Survey are displayed in Table 3. There was no effect ($P = 0.37$) of $LSCC_C$ at peak lactation for MSU ewes, and results

presented in Table 3 reflect the final model when $LSCC_C$ at early lactation (nested within production year) was fit. Within the MSU flock, LW120 was greater ($P = 0.005$) in 2017 than 2018. Additionally, first parity MSU ewes had lighter ($P \leq 0.001$) LW120 than second and third parity ewes, which were not different ($P = 0.99$). However, LW120 between MSU Targhee and Rambouillet were similar ($P = 0.64$). The linear covariate of $LSCC_C$ on LW120 was significant and negative in 2017 ($P = 0.01$) but not in 2018 ($P = 0.11$) for MSU ewes.

The final model for LW120 in USSES Survey ewes included main classification effects and the linear covariate of $LSCC_C$ at peak lactation (nested within production year). Total litter weight adjusted to 120 d tended to be greater in 2018 than 2017 ($P = 0.09$). First and second parity USSES Survey ewes had similar LW120 ($P = 0.23$), but both were lighter than third parity ($P \leq 0.01$). Additionally, terminal composite ewes were higher performing ($P < 0.001$) than Suffolk. As seen in MSU ewes, the linear covariate of $LSCC_C$ on LW120 for USSES Survey ewes was significant and negative in 2017 ($P = 0.003$) but not in 2018 ($P = 0.10$).

Peak lactation $LSCC_C$ did not impact LW120 in USSES Control ewes ($P = 0.63$). However, the effect of early lactation $LSCC_C$ was not dependent on ewe breed or production year and, when fit independent of these effects, negatively impacted LW120 (-9.18 ± 0.02 kg/unit $LSCC_C$; $P = 0.02$). In this model, LW120 were similar ($P = 0.12$) for Rambouillet (34.4 ± 2.13 kg), Suffolk (36.3 ± 4.06 kg), and Targhee ewes (28.6 ± 2.23 kg). Additionally, LW120 was not influenced by production year ($P = 0.97$) for USSES Control ewes.

Udder and Teat Morphometry

Descriptive statistics of udder and teat morphometric traits for MSU and USSES surveys are displayed in Table 4. Within MSU ewes, mean TL were similar in early and peak lactation. The means for UL, UC, and DS decreased while TP increased from early to peak lactation in MSU ewes. While not tested formally, and likely dependent on ewe breed and age, mean TL were similar between MSU and USSES Survey ewes in peak lactation while other udder morphometry traits were generally greater in USSES Survey ewes.

Left and right TL were strongly correlated within lactation stage for MSU (0.81-0.83) and USSES Survey ewes (0.77; $P < 0.001$). Estimated Pearson correlation coefficients between additional udder and teat traits for MSU and USSES Survey ewes are displayed in Tables 5 a and b, respectively. Within traits for MSU ewes, UC (0.63), UL (0.46), TP (0.51), DS (0.47), and TL (0.51; $P < 0.01$) were moderately correlated between early and peak lactation. Across traits, UC and UL were strongly correlated within early (0.80) and peak lactation (0.85; $P < 0.01$). Within peak lactation, TP was moderately and negatively correlated with UL (-0.49) and UC (-0.53; $P < 0.001$). Estimated correlation coefficients between traits across lactation were generally low, with the exception of TP at early and UL at peak lactation (-0.40; $P < 0.001$). Within USSES Survey ewes, the greatest estimated correlation coefficients were between UL and UC (0.52) and between TL and TP (0.37; $P < 0.001$), which were still moderate in magnitude.

Factors Affecting Ewe SCC and Productivity

The traits collected in Table 1 were first averaged across lactation for MSU ewes, then expressed in categories to predict $LSCC_C$ in early and peak lactation for MSU and USSES Survey ewes, respectively. The most parsimonious model to predict $LSCC_C$ at early lactation for MSU ewes included main effects of production year, TL, EDT, SYM, and SNT ($R^2 = 0.51$; adjusted $R^2 = 0.44$). Within USSES Survey ewes, parity, production year, and SNT all were included in the final model to predict $LSCC_C$ at peak lactation ($R^2 = 0.27$; adjusted $R^2 = 0.25$).

Least-squares means of main effects on $LSCC_C$ for MSU and USSES Survey ewes are displayed in Table 6 a and b, respectively. Average udder-half LSCC in peak lactation was greater ($P = 0.003$) in 2018 than 2017 for MSU. However, effects of production year cannot be replicated or observed in the future. Of the traits that can, TL, EDT, and SNT significantly contributed ($P \leq 0.003$) to differences in $LSCC_C$ at early lactation for MSU ewes. Interestingly, MSU ewes with EDT scores of 1 or greater across lactation had lower $LSCC_C$ in early lactation ($P = 0.003$) than ewes with no teat damage (EDT = 0). Additionally, the presence of SNT was associated with reduced $LSCC_C$ in early lactation ($P < 0.001$). Early lactation $LSCC_C$ also generally increased with TL and was lowest for ewes with TL < 3 cm compared to those with TL > 3.3 cm ($P \leq 0.02$). Production year also influenced $LSCC_C$ in peak lactation for USSES Survey ewes and was greater ($P < 0.001$) in 2018 than 2017. The only other replicable and significant main effect that contributed to $LSCC_C$ in peak lactation for USSES Survey ewes was parity, where 1-yr-old ewes had greater ($P = 0.01$) $LSCC_C$ than 3-yr and older ewes.

DISCUSSION

Relationship Between Udder Health and Ewe Productivity

Somatic cells are primarily leukocytes produced by the immune system in response to inflammation or infection (Lee and Outteridge, 1981), and SCC is commonly used to infer subclinical mastitis status in dairy ewes (González-Rodríguez et al., 1995; Suarez et al., 2002; Riggio et al., 2013). For the majority of the production year, lactating dairy ewes are handled on a daily basis, and milk samples to estimate SCC on an individual or group are easily obtained. Past research has reported that milk yield was 3 to 54% less in subclinically infected dairy ewes (Dario et al., 1996; Saratsis et al., 1999; Winter et al., 2003). If similar relationships between SCC, subclinical mastitis, and milk production is realized in non-dairy ewes, it is likely that lamb growth and survival is negatively impacted. In non-dairy ewes in Norway, Holmøy et al. (2014) reported that 7 and 10% clinically infected ewes displaying moderate to severe symptoms lost lambs within 5 d of lambing, respectively. Gross et al. (1978) and Ahmad et al. (1992a) reported reductions in ADG by 5 and 11%, respectively, in lambs reared by subclinically infected ewes. Additionally, lambs reared by subclinically infected ewes were reported to consume 28% more creep feed than lambs reared by healthy ewes (Fthenakis and Jones, 1990b). In the present study, negative effects of $LSCC_C$ on 120 d litter weight were detected in all 3 extensively managed flocks in the Western U.S., which is in general agreement with the reviewed literature in other management systems of non-dairy ewes.

In an abattoir survey of culled ewes, 50% of udders were classified as abnormal, albeit researchers could not determine that the abnormalities were the primary cause of

culling by the producer (Herrtage et al., 1974). However, Watson and Buswell (1984) reported that udder abnormalities accounted for 46% of cull ewes in a typical UK lowland flock. Intramammary masses (IMM) are perceived as an udder abnormality, are relatively common in meat- and wool-type ewes (3-30%; Grant et al., 2016; Crump et al., 2018; Griffiths et al., 2019a), and have been identified as a major cause of ewe culling (Petridis and Fthenakis, 2014). It is likely that IMM impair mammary function, which can have negative consequences for lamb health and growth. For example, Griffiths et al. (2019a) reported that ewe IMM were associated with greater odds of failure of a lamb to survive until weaning (1.6-4.0 times) and reduced lamb growth rate (0.4-10.9%). In the present study, IMM was assessed as an udder palpation score (PALP) but was not a significant contributor to predict early or peak lactation LSCC in MSU or USSES Survey ewes.

Teat lesions have also been shown to be present in 1-4% of ewes surveyed (Madel, 1981; Sulaiman and Al-Sadi, 1992). Huntley et al. (2012) estimated that lambs reared by ewes with traumatic teat lesions weighed 1 kg less than lambs reared by healthy ewes 2 wk after the lesion was observed. They attributed this to ewes limiting their lamb(s) from suckling until the wound was healed. Additionally, Grant et al. (2016) reported ewes with traumatic teat lesions reared lambs with slightly reduced ADG (-20 g/d). However, past reports have indicated minimal direct impact of lesions on ewe subclinical mastitis or SCC (Watkins et al., 1991; Huntley et al., 2012). In the present study, the presence of teat lesions was a significant contributor to predict early lactation $LSCC_C$ in MSU ewes. Interestingly, the model estimates indicated that $LSCC_C$ was lower

(0.23 $\log_{10}$ units) in ewes with some degree of external teat damage during early and lactation. One possible explanation for this could be that, at the time of sampling, the teat lesion was in the later stages of healing and the immune response was lessened from the initial trauma. Still, this doesn't explain why ewes with visually healthy teats would have greater $LSCC_C$, and this result could likely be an artifact of the data.

Most ewes have 2 functional teats, although there is evidence that the presence of SNT is under genetic control (Peng et al., 2017). Past observations of ewes with SNT having a greater proportion of twins than ewes with 2 teats led Bell (1904) to postulate that there may be a correlation between fertility and presence of SNT. However, this has not been validated using modern models for genetic prediction. Maijala and Kyle (1988) reported that 17-20% of Finnsheep ewes sampled had SNT and, while milk yield from SNT ranged from 0 to 170 mL, 47-87% of SNT from 4-teated ewes produced no milk. No past reports investigating the relationship between SNT and ewe mastitis incidence were found. Results from the present study indicated the presence of SNT was associated with an 8% reduction in $LSCC_C$ during early lactation for MSU ewes. However, SNT did not effect $LSCC_C$ in peak lactation for USSES Survey ewes. Therefore, the association between SNT and subclinical mastitis should be further validated before major conclusions can be drawn.

Linear scoring systems have been developed for dairy sheep breeds to evaluate and assess individuals on a combination of udder and teat characteristics. Researchers have estimated moderate to high repeatabilities (0.51-0.86) of similar linear scores within a single lactation in dairy ewes (de la Fuente et al., 1996; Fernández et al, 1997; Casu et

al., 2006). Additionally, moderate to strong positive phenotypic correlations were also estimated between different udder morphometric traits (0.40-0.82). In dairy ewes, udder circumference and width have been associated with milk yield and explain up to 45% of its variation (Kominakis et al., 2009). However, Fernández et al. et al. (1995) reported insignificant contributions of teat position, angle, length and width on milk yield. Additionally, Kominakis et al. (2009) found no measured teat characteristics predicted SCC in dairy ewes. In non-dairy ewes, positive and moderate phenotypic correlations have been estimated between teat placement and udder width (0.26), teat placement and udder depth (0.35), and udder depth and length (0.63; Cooper et al., 2013; McLaren et al., 2018). In the present study, the strongest estimated correlations within MSU ewes within a collection time were between UC and UL at early lactation (0.80) and, during peak lactation, UC and UL (0.85), TP and UC (-0.53), and UL and TP (-0.49). While average TL was associated with $LSCC_C$ in early lactation for MSU ewes, most udder and teat measurements did not influence $LSCC_C$ in either flock.

Implications

Results indicated that increasing somatic cell count was generally associated with reduced ewe productivity. Past research in these flocks reported that somatic cell count thresholds corresponding to ewe intramammary infection ranged from 250 x 10^3 to 1700 x 10^3 cells/mL. On average, ewes sampled in the present study with a somatic cell count of 500 x 10^3 cells/mL were expected to wean 4.6-7.4 kg less lamb than ewes with a somatic cell count of 100 x 10^3 cells/mL. This represents a potential economic loss of $19-31 per ewe per year assuming a national average feeder lamb value of $4.18/kg

(USDA-AMS). Additionally, 24-31% of ewes sampled recorded a somatic cell count of 500 x 10^3 cells/mL or greater. However, the relationship between ewe somatic cell count and productivity was dependent on lactation stage, flock, and year. Furthermore, phenotyping somatic cell count is cost-prohibitive in extensively managed ewe flocks. Indicator traits could be applied as selection criteria for reducing subclinical mastitis in such flocks. While significant correlations were detected among many udder and teat morphometric traits, most were not consistently predictive of ewe somatic cell count. Therefore, additional studies investigating effective methods of reducing mastitis in meat- and wool-type ewes are warranted.

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Table 1. Description of udder and teat morphometric, health, and anatomical traits collected from Montana State University and U.S. Sheep Experiment Station ewes.

Trait	Units	Description
TL	cm	Average length of left and right teats; measured from base to distal end.
UL	cm	Udder length; measured from udder cleft to abdominal wall along mid-line.
UC	cm	Udder circumference; measured around udder at widest point.
TP	1 – 9	Teat placement; adapted from Casu et al. (2006)
DS	1 – 9	Degree of separation of udder halves; adapted from Casu et al. (2006)
SYM	0 – 2	Udder symmetry (0 = symmetrical; 1 = slightly unsymmetrical; 2 = very unsymmetrical); observed from animal rear.
PALP	0 – 2	Udder palpation score (0 = no intramammary masses; 1 = single intramammary mass; 2 = multiple intramammary masses); quantified from palpating udder halves.
EDT	0 – 2	External damage to teats (0 = no damage; 1 = small abrasion, chapped skin, or few warts; 2 = large abrasions, multiple warts).
SNT	0, 1	Supernumerary teats (0 = absent; 1 = present); observed from ewe in sitting position.
UWS	1 – 3	Udder wool score (1 = little or no wool coverage; 2 = moderate wool coverage; 3 = heavy wool coverage); quantified from images of udder of ewe in sitting position.
BCS	1 – 5	Body condition score assessed by palpating the spinous and transverse processes for degree of subcutaneous adipose tissue.

Table 2. Estimated Pearson correlation coefficients between udder half and composite $\log_{10}$ somatic cell count of milk samples collected from Montana State University ewes at early and peak lactation.

Trait[1]	$LSCC_{L,E}$	$LSCC_{C,E}$	$LSCC_{R,P}$	$LSCC_{L,P}$	$LSCC_{C,P}$
$LSCC_{R,E}$	0.50*	0.85*	0.21	0.05	0.12
$LSCC_{L,E}$	-	0.88*	0.32*	0.19	0.23*
$LSCC_{C,E}$	-	-	0.31*	0.15	0.21
$LSCC_{R,P}$	-	-	-	0.92*	0.98*
$LSCC_{L,P}$	-	-	-	-	0.98*
$LSCC_{C,P}$	-	-	-	-	-

[1]LSCC = $\log_{10}$ transformed somatic cell count of right half (R), left half (L), and average udder half (C) collected at early (E) and peak (P) lactation.

*Estimated Pearson correlation coefficient is different from zero ($P \leq 0.05$).

Table 3. Least-squares means (±SE) of main effects and solutions for the linear covariate of log_{10} transformed average udder half somatic cell count ($LSCC_C$) on 120 d adjusted litter weaning weight for Montana State University (MSU) and U.S. Sheep Experiment Station (USSES) Survey ewes.

		Flock, kg	
Effect	Level	MSU	USSES Survey
Year	2017	39.7 ± 1.47[a]	47.7 ± 2.42
	2018	37.8 ± 1.89[b]	53.9 ± 3.50
Parity[1]	1	31.9 ± 1.76[b]	40.3 ± 5.58[b]
	2	41.9 ± 2.02[a]	50.8 ± 3.40[b]
	3+	42.4 ± 2.01[a]	61.3 ± 2.02[a]
Breed[2]	Rambouillet/Suffolk	39.3 ± 1.78	44.7 ± 2.42[b]
	Targhee/TC	38.2 ± 1.42	56.9 ± 3.25[a]
$LSCC_C$ (year)[3]	2017	-9.6 ± 3.70[*]	-14.7 ± 4.86[*]
	2018	ns	ns

[1]MSU and USSES ewes were 2- and 1-yr of age at first parity, respectively.
[2]Rambouillet and Targhee ewes were sampled at MSU; Suffolk and terminal composite (TC; ⅜ Suffolk, ⅜ Columbia, ¼ Texel) were sampled at USSES.
[3]$LSCC_C$ at early lactation (< 5 d) is fit for MSU ewes; $LSCC_C$ at peak lactation (30-45 d) is fit for USSES ewes.
[a,b]Means within a column and effect with no superscript in common are different ($P \leq 0.001$).
[*]The solution for the linear covariate of $LSCC_C$ nested within production year is different from zero ($P \leq 0.01$).
[ns]The solution for the linear covariate of $LSCC_C$ nested within production year is not different from zero ($P \geq 0.10$).

Table 4. Descriptive statistics of udder and teat morphometry traits within lactation stage for Montana State University (MSU; n = 74) and U.S. Sheep Experiment Station (USSES; n = 157) Survey ewes.

			Descriptive statistic			
Flock	Stage[1]	Trait[2]	Min.	Max.	Mean	SD
MSU	Early	TL, cm	2.22	4.76	3.27	0.64
		UL, cm	19.05	36.20	25.85	3.81
		UC, cm	36.83	62.23	48.66	5.85
		TP	1	6	3.54	1.09
		DS	1	6	2.42	1.12
	Peak	TL, cm	2.22	4.92	3.29	0.56
		UL, cm	15.24	30.48	22.38	3.62
		UC, cm	26.67	57.15	44.22	6.38
		TP	2	7	4.15	1.35
		DS	1	5	2.14	0.91
USSES Survey	Peak	TL, cm	2.22	4.76	3.28	0.50
		UL, cm	18.42	49.53	26.93	4.12
		UC, cm	38.1	66.04	49.25	5.12
		TP	2	8	4.29	1.42
		DS	1	6	2.53	1.00

[1]Early = < 5 d post-lambing; Peak = 30-45 post-lambing.

[2]TL = teat length; UL = udder length, UC = udder circumference, TP = teat placement, DS = degree of separation between udder halves.

Table 5a. Estimated Pearson correlation coefficients between udder and teat morphometry traits collected at early and peak lactation on Montana State University ewes.

Trait[1]	UL_E	UC_E	TP_E	DS_E	TL_P	UL_P	UC_P	TP_P	DS_P
TL_E	0.30*	0.27*	0.17	0.28*	0.51*	-0.01	-0.04	0.23*	0.04
UL_E	-	0.80*	0.01	0.10	0.12	0.46*	0.42*	0.02	0.12
UC_E	-	-	-0.14	0.17	-0.04	0.53*	0.63*	-0.17	0.22
TP_E	-	-	-	-0.06	0.33*	-0.40*	-0.30*	0.51*	0.02
DS_E	-	-	-	-	0.03	0.03	0.07	0.11	0.47*
TL_P	-	-	-	-	-	-0.07	-0.18	0.29*	-0.05
UL_P	-	-	-	-	-	-	0.85*	-0.49*	0.05
UC_P	-	-	-	-	-	-	-	-0.53*	0.08
TP_P	-	-	-	-	-	-	-	-	0.23*

[1]TL = average teat length; UL = udder length; UC = udder circumference; TP = teat placement; DS = degree of separation between udder halves; collected at early (< 5 d; E) or peak (30-45 d; P) lactation. A description of traits is provided in Table 1.

*Estimated Pearson correlation coefficient is different from zero ($P \leq 0.05$).

Table 5b. Estimated Pearson correlation coefficients between udder and teat morphometry traits collected at peak lactation on U.S. Sheep Experiment Station Survey ewes.

Trait[1]	UL_P	UC_P	TP_P	DS_P
TL_P	0.15	0.10	0.37*	-0.23*
UL_P	-	0.52*	-0.06	-0.09
UC_P	-	-	-0.01	0.13
TP_P	-	-	-	-0.21*

[1]TL = average teat length; UL = udder length; UC = udder circumference; TP = teat placement; DS = degree of separation between udder halves; collected at peak (30-45 d; P) lactation. A description of traits is provided in Table 1.

*Estimated Pearson correlation coefficient is different from zero ($P \leq 0.01$).

Table 6a. Least-squares means (±SE) for the main classification effects identified in model selection procedures on average udder half $\log_{10}$ transformed somatic cell count in early lactation (< 5 d; $LSCC_{C,E}$) for Montana State University ewes.

Effect[1]	Level	$LSCC_{C,E}$
Year	2017	5.23 ± 0.08^{b}
	2018	5.50 ± 0.07^{a}
TL, cm	< 3	5.11 ± 0.09^{b}
	3 – 3.3	$5.34 \pm 0.09^{a,b}$
	3.3 – 3.6	5.53 ± 0.09^{a}
	> 3.6	5.47 ± 0.09^{a}
EDT	0	5.48 ± 0.07^{a}
	1+	5.25 ± 0.07^{b}
SYM	0	5.44 ± 0.05
	1+	5.29 ± 0.10
SNT	Absent	5.57 ± 0.05^{a}
	Present	5.15 ± 0.09^{b}

[1]TL = average teat length; EDT = maximum external teat damage; SYM = maximum udder symmetry score; SNT = presence of supernumerary teats.

[a,b]Means within an effect with no superscript in common are different ($P \leq 0.02$).

Table 6b. Least-squares means (±SE) for the main classification effects identified in model selection procedures on average udder half $\log_{10}$ transformed somatic cell count in peak lactation (30-45 d; $LSCC_{C,P}$) for U.S. Sheep Experiment Station Survey ewes.

Effect[1]	Level	$LSCC_{C,P}$
Year	2017	5.33 ± 0.05^{b}
	2018	5.80 ± 0.06^{a}
Parity	1	5.77 ± 0.11^{a}
	2	$5.50 \pm 0.07^{a,b}$
	3+	5.42 ± 0.04^{b}
SNT	Absent	5.52 ± 0.05
	Present	5.61 ± 0.06

[1]SNT = presence of supernumerary teats.

[a,b]Means within an effect with no superscript in common are different ($P \leq 0.01$).

CHAPTER FIVE

CONCLUSION

Results from the first study indicated intramammary infection was a common disease within the Montana State University (MSU) and U.S. Sheep Experiment Station (USSES) flocks. Milk samples collected from ewes which appeared to be otherwise healthy commonly had a variety of potentially pathogenic bacteria isolated from them. However, the frequency of intramammary infection varied across flocks, years, and lactation stages. In the reviewed literature, *Staphylococcus*, *Streptococcus*, *Bacillus*, *Mannheimia*, and *Escherichia* species were among the most common pathogens isolated in past research of subclinical mastitis in non-dairy ewes. In the first study, no *Mannheimia* spp. were isolated, and *Streptococcus* spp. were isolated at low frequencies. Nonetheless, *E. coli*, *Staphylococcus* spp., and *Bacillus* spp. were among the most common bacteria isolated from milk samples. In dairy animal research and production, somatic cell count thresholds are used to infer intramammary infection, but there is not a clear threshold in wool- or meat-type ewes. The results of the first study indicate milk somatic cell count can be used to infer intramammary infection, albeit thresholds are variable across flocks and lactation stage.

Results from the second study indicated that increased somatic cell counts were generally associated with reduced ewe productivity, assessed as 120 day adjusted litter weaning weight. On average, ewes in this study with a somatic cell count of 500×10^3 cells/mL were expected to wean litters which weighed between 4.6 and 7.4 kg less than

ewes with a somatic cell count of 100 x 10^3 cells/mL. This loss of production represents a potential economic loss of between $19 and 31 per ewe (USDA-AMS). Between 24 and 31% of sampled ewes recorded a somatic cell count greater than 500 x 10^3 cells/mL, which indicates elevated counts are common within a flock and the associated economic loss is great.

Across flocks, 28% of sampled ewes had somatic cell counts greater than 500,000 cells/mL and were expected to wean litters that weighed 6 kg less than litters reared by ewes with a somatic cell count of 100,000 cells/mL. If a 500-ewe flock is assumed, 140 ewes would have a somatic cell count greater than 500,000 cells per mL and result in a flock loss of 840 kg in lamb weaning weight. The national average market lamb price during 2017 and 2018 in the U.S. was $4.18/kg, therefore this loss in ewe productivity represents an economic loss of $3511 per year. If a husbandry practice were able to reduce the frequency of ewes with elevated somatic cell counts greater than 500,000 cells/mL to 20 or 10% of the flock, then an additional 240 kg ($1003) or 540 kg ($2257) could be expected. However, such cost- and labor-effective treatments are still unknown.

The association between ewe somatic cell count and productivity varied with flock, production year, and lactation stage. Furthermore, quantifying somatic cell count in an extensive production system is cost-prohibitive. Therefore, an objective of the second study was to identify indicator traits which could be applied as a selection criterion for reducing subclinical mastitis in such flocks. Although significant correlations were estimated among many udder and teat morphometric traits, most were not consistently predictive of ewe somatic cell count. In conclusion, the results from the first and second

studies indicate that subclinical mastitis is a common and costly disease, and future research investigating cost-effective husbandry practices to reduce its production impacts and prevalence is warranted.

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