Longitudinal Studies of Intramammary Infection in Suckler Ewes

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Table of Contents

List of Tables

List of Figures

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This thesis is dedicated to Christine, Irene and Paul for their love and support.

Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy in Veterinary Epidemiology. It has been composed by myself and has not been submitted in any previous application for any other degree.

The research work described in Chapter 3 was published as a paper in the Journal of Dairy Science in September 2012 (Huntley *et al.*, 2012). The research work described in Chapters 4 and 5 are in the process of being written up for publication in a peer reviewed journal. The work in Chapter 2 will be written up for publication in a peer-reviewed journal subject to results from further bacteriological work which is in progress at the University of Warwick.

The declare that I conducted all of the field work and collected all of the data, with the exception of udder conformation measurements and teat lesion observations in Chapters 3 and 4 which were conducted by Selin Cooper and observations of clinical mastitis in Chapter 5 which were recorded by Frank Hall.

Summary

Four longitudinal studies were conducted. The first study investigated the longitudinal pattern of udder half somatic cell count (HSCC) and intramammary infection (IMI) in 48 UK suckler ewes over the first 10 weeks of lactation. This was the first study to demonstrate that HSCC of suckler ewes followed a quadratic and cubic relationship with days in lactation over the first 10 weeks of lactation. Udder half somatic cell count was also explained by presence of bacteria. Ewes older than 6 years of age had significantly higher HSCC than younger ewes.

The second study investigated the relationships between udder conformation, SCC and lamb weight. Whilst accounting for lamb age and birth weight, significantly lower lamb weight was associated with a ewe SCC of >400,000 cells/ml (-1.7 kg), a traumatic teat lesion (bite, tear or chapping) 2 weeks previously (-1.1 kg), and a ewe body condition score (BCS) of <2.5 before lambing (-1.3kg). Higher HSCCs were observed in ewes with a lower suspended udder, and older ewes in poorer body condition. The findings from this study make an important contribution to the knowledge of the impact of udder health of suckler ewes by demonstrating that udder conformation is associated with IMI and that IMI and teat damage are negatively associated with lamb weight.

The third study investigated the effect of dry cow therapy (DCT) on subclinical mastitis in a lowland flock with a low incidence of clinical mastitis by recording HSCC and lamb weights in the following lactation. To the author's knowledge, there are no published reports of the effect of broad spectrum DCT on subclinical mastitis in suckler ewes in the literature. No significant effect was found between the use of DCT and HSCC or lamb weight in the subsequent lactation.

The fourth study was a randomised controlled trial to assess the effect of DCT on clinical mastitis in a suckler flock with a high level of clinical mastitis. Dry cow treatment significantly reduced the incidence of clinical mastitis over one year, with a 70% reduction of clinical mastitis in ewes that received treatment from 6.2% to 1.8%. This was the first field trial to investigate and demonstrate the clinical benefit of the use of a broad spectrum DCT in suckler ewes.

These studies have enhanced our knowledge of longitudinal patterns of infection and demonstrated the importance of udder health for optimising production of suckler ewes. Factors to control for when using SCC as a tool to measure intramammary infection were described.

Abbreviations

ADAS	Agricultural Development and Advisory Service
AGID	agar gel immunodiffusion test
ANOVA	analysis of variance
BCS	body condition score
CFU	colony forming units
CI	confidence interval
CMT	California mastitis test
CNS	coagulase negative Staphylococcus spp.
CV	coefficient of variation
DCT	dry cow therapy
df	degrees of freedom
DIL	days in lactation
EBLEX	English Beef and Lamb Executive
HSCC	udder half somatic cell count
IMI	intramammary infection
kg	kilogram
LW	lamb weight
ml	millilitre
MS	mean square
Obs	observations
QMMS	Quality Milk Management Services LTD
QMS	Quality Meat Scotland
PFB	partial farm budget
PBS	phosphate buffered saline
SAC	Scottish Agricultural College
SCC	somatic cell count
sf	significant figures
Spp.	species
SS	sum of squares
Std. Dev	standard deviation
Std. Err	standard error
SPVS	Society of Practicing Veterinary Surgeons
WT	Whiteside test

1. CHAPTER 1: GENERAL INTRODUCTION

This introduction provides a background to intramammary infection in suckler (meat) ewes. The definition of mastitis, detection of intramammary infection (IMI), bacterial species associated with IMI, risk factors for IMI and impacts of IMI on suckler ewe health and production are presented.

1.1 Definitions of mammary gland disease

Mastitis is inflammation of the udder that usually develops as a result of IMI. Intramammary infection is the invasion and multiplication of potentially pathogenic micro-organisms, usually bacteria, in the mammary gland. An immune response in the mammary gland usually follows infection, such that the number of leucocytes in the affected gland increases and clinical mastitis may result (Albenzio *et al.*, 2002).

Clinical mastitis is externally evident visually or by palpation of the udder (Saratsis *et al.*, 1998) or by systemic effects such as pyrexia (Conington *et al.*, 2008) or altered behaviour of the affected animal (Calavas *et al.*, 1997). Farmers of suckler ewes usually diagnose clinical mastitis using visual signs and tactile signs. Visual signs include an increase or decrease in size of the affected mammary gland in comparison to the contralateral unaffected gland, subcutaneous lumps or an unusual discharge upon manual expression of the mammary gland (Marogna *et al.*, 2010). Colour changes may be apparent (Calavas *et al.*, 1998) or the mammary gland may appear necrosed (Omaleki *et al.*, 2011). The ewe may exhibit one or more signs of lethargy, pyrexia, inappetence, pain, apparent lameness, resentment of udder palpation or will not let the lamb suckle (Winter, 2001). Tactile signs are that, upon palpation, the mammary gland may feel hard or lumpy and abnormally hot or cold, indicative of local vascular insult. Manual expression of milk may be difficult or non-productive.

Although the true point of infection is rarely known, clinical mastitis is typically classified according to the speed of appearance of clinical signs post infection, as per-acute, acute or chronic (Morogna *et al.*, 2010) and may be severe or mild.

Subclinical mastitis is infection of the mammary gland with no outward clinical signs of inflammation. It has been defined as the simultaneous bacterial isolation and presence of an inflammatory reaction in the mammary gland such that functional changes occur in the absence of abnormal gross findings in the mammary gland or systemically (Gougoulis *et al.*, 2008). Despite a lack of overt clinical signs a reduced milk yield is frequently observed in dairy cows, dairy goats and dairy sheep with subclinical mastitis (Albenzio *et al.*, 2002). In contrast, in suckler ewes, subclinical mastitis will often pass unnoticed, although a decreased lamb weaning weight has been recorded (Fthenakis and Jones., 1990; Arsenault *et al.*, 2008).

Whilst clinical cases may be considered the tip of the iceberg in terms of infection, it is probable that, in many instances, sub-clinical, acute clinical and chronic clinical mastitis are just different presentations of the same infection. In a study of subclinical mastitis in lowland suckler ewes on seven farms in Southern England, 7% of ewes had sub-clinical mastitis 2-3 weeks after lambing and, of the ewes that subsequently developed clinical mastitis, almost a third of the glands previously had subclinical mastitis with the same organism diagnosed at a previous sampling event (Watkins *et al.*, 1991).

1.2 Detection of intramammary infection in the udder

The increase in leucocytes in response to bacterial IMI is measured as an increase in milk somatic cell count (SCC) which is the number of somatic cells per millilitre of milk. The vast majority of somatic cells in milk are leucocytes, predominantly

neutrophils (Lafi, 2006; Albenzio *et al.*, 2002). The somatic cell count of milk is thus often used as a proxy indicator of both clinical and subclinical mastitis (Sordillo *et al.*, 1997; MacDougall *et al.*, 2002). In bovine dairy production, somatic cell counting is the most established, relatively low cost, practical method (Schukken *et al.*, 2003) to monitor udder health and milk quality and has long been used as an indicator of IMI not just at the herd level (bulk milk) (Barkema *et al.*, 1998) and cow level (Green, *et al.*, 2006) but also, in research, at the individual gland level (Peeler *et al.*, 2002; Green, *et al.*, 2004). Whilst SCC is widely used as an indicator of IMI in commercial dairy ruminants (Schukken *et al.*, 2003; Peeler *et al.*, 2003), it is rarely employed as a method for investigating udder disease in commercial suckler ewes, other than for research purposes (Clements *et al.*, 2003).

SCC is quantified in the laboratory via finely calibrated automated processes using a Fossameter (Burriel, 1997; Paape *et al.*, 2007; Pengov, 2001) or Coulter counter (Dohoo *et al.*, 1984) or, less frequently, manual counting by direct microscopy (Mavrogianni and Fthenakis, 2007). Somatic cell count may also be assessed by the use of ordinal score-based pen-side tests such as the California mastitis test (CMT) or the Whiteside test (WT). These are indirect methods using reagents and are often used by farmers in the dairy industry in order to quickly diagnose IMI in an individual dairy animal (Sargeant *et al.*, 2001). Such pen-side methods have been frequently used to measure SCC in research of IMI of suckler sheep (Lafi., 2006; Fragkou *et al.*, 2008; Watkins *et al.*, 1991). A moderately good agreement between automated quantitative laboratory measurement of SCC with CMT and WT has been demonstrated in dairy cows (Sargeant *et al.*, 2001) and dairy sheep (Fthenakis, 1995; Lafi, 2006). However, agreement is variable in studies of suckler sheep: Gonzalez-Rodriguez and Carmenes (1996) and Clements *et al.* (2003) reported a good

agreement (r=0.82) between CMT and a SCC > 300,000 cells/ml, whilst Keisler *et al.* (1992) reported a lower correlation (r=0.58) with SCC and CMT compared to SCC with direct microscopic somatic cell counting (r=0.95).

The "cut off level" of SCC for which a case of subclinical mastitis may be presumed in sheep has been defined differently by different authors (Fthenakis, 1991; Berthelot *et al.*, 2006; Pengov, 2001). Subclinical mastitis has been defined in British suckler ewes by Fthenakis (1991) and in Jordanian dairy ewes by Lafi *et al.* (1998) as a mammary gland SCC > 1 x 10^6 cells/ml, whereas other sources consider most healthy glands of European dairy ewes as < 250 x 10^3 cells/ml (Pengov, 2001) or < 500 x 10^3cells/ml (Berthelot *et al.*, 2006). In the UK an individual cow cell count of 200,000 cells/ml is considered high (Madouasse *et al.*, 2012) and indicative of IMI whilst those with SCC <100,000 cells/ml considered healthy (Bradley and Green, 2005). The current EU cut off for liquid milk for commercial sale for the production of raw and heat treated milk products from dairy cows is a three month geometric mean of 400 x 10^3 cells/ml (EC Council Directive 92/46/EEC). In contrast to cows, there is very little regulation of SCC in commercially produced ewe milk, rather the emphasis is solely on the bacterial plate count of milk.

Similarly, for the penside CMT and WT, the recommended cut off for definition of subclinical mastitis varies according to author and sheep production type. A study by Fthenakis (1994) revealed that, in dairy ewes, a CMT cut off of Score 1 gave an accuracy of 93% when the prevalence of subclinical mastitis in the flock was 10-25%. Lafi (2006) demonstrated a sensitivity of over 90% and a specificity of 79% of CMT for the detection of subclinical mastitis in dairy sheep but that these values changed according to different stages of lactation with specificity falling to 59% in

the third stage of lactation. For suckler ewes, Clements *et al.* (2003) suggested a score 3 as the cut off level for subclinical mastitis. In a study using the WT to indicate SCC level of suckler ewes in Southern England, a WT score of 1 or above was considered to be indicative of subclinical mastitis in milk samples that were also positive for bacterial culture. As with CMT, specificity was not perfect and positive WT scores of milk samples that were bacteriologically negative were observed. The authors noted that lower specificity could have arisen due to limited culture techniques resulting in a false negative bacteriology result (Watkins *et al.*, 1991). An alternative explanation is that infection had already been cleared yet inflammation had persisted.

1.3 Bacteria associated with intramammary infection

Bacterial culture and molecular techniques, such as polymerase chain reaction (PCR), are the most commonly used methods to identify the presence and species of bacteria in a sample of milk or mammary gland secretion (Taponen *et al.*, 2009; Viguier *et al.*, 2009). Although not commonly performed in suckler ewes in a non-research setting, such approaches are routinely used in commercial dairy cows for the investigation of IMI and indeed bulk milk is monitored for the presence and levels of pathogenic bacteria under EC Council Directive 92/46/EEC. This stipulates a bacterial plate count for dairy cows of <100,000 bacteria/ml for the sale of milk products to be heat treated or sold raw and limits of 1, 000,000 and 500, 000 bacteria/ml for dairy ewe milk products to be heat treated and sold raw respectively. In addition, for both dairy cows and dairy ewes, low levels of *Staphylococcus aureus* are necessary for sale of bulk milk for raw milk products.

The definition of IMI using bacterial culture is dependent on bacterial species and load. In a study of subclinical mastitis in dairy ewes and goats in the USA, an IMI infection was defined as a threshold of >500 cfu/ml of each of 1 to 3 colony types (McDougall *et al.*, 2002). In another study, ewes' udder halves with >250cfu/ml were classified as having IMI with any bacterial species except for *Staphylococcus aureus* where a lower limit for of >50cfu/ml was considered as infected (Ariznabarreta *et al.*, 2002). In dairy cows one definition of IMI is milk culture of >1 x 10^2 cfu/ml for a contagious pathogen or >2 x 10^2 for an environmental pathogen (Barkema *et al.*, 1998).

Bacterial species associated with IMI depend on ruminant species and production type. In some countries, particularly in Southern Europe, the Middle East and parts of the developing world, ewes are of mixed production type and suckle lambs in the early rearing period, before being machine or hand milked. In contrast, ewes in the UK are suckler ewes or dairy ewes but not both. Exposures to different bacteria may thus vary considerably between production types. The effect of different production systems must be considered when interpreting sheep studies from different countries although whether study ewes are those used solely for dairy or meat production, or for both, is not clearly and consistently declared in the literature.

In both dairy and suckler ewes, the most commonly isolated bacteria associated with subclinical IMI or subclinical mastitis are Coagulase Negative *Staphylococcus* spp. (CNS) (Kirk *et al.*, 1996; Berthelot *et al.*, 2006; Fthenakis, 1994; Mavrogianni *et al.*, 2007). In a study of suckler ewes in Southern England, of udder half milk samples that were bacteriologically positive but not Whiteside positive and so were considered to have IMI but not subclinical mastitis, the predominant bacterial isolate

was CNS (53%). In the same study, CNS was the second most common isolate (33%) from udder half milk samples that were bacteriologically positive and Whiteside test positive and therefore classified as having subclinical mastitis, although *Streptococcus* spp. was the predominate isolate (42%). Other bacteria isolated but with lower frequency were *M. haemolytica* (17%) and *S. aureus* (8%) (Watkins *et al.*, 1991). Coagulase Negative *Staphylococcus* spp. are an uncommon cause of clinical mastitis in sheep (Mørk *et al.*, 2007). Similarly, in dairy cows, CNS spp. are also commonly associated with IMI and less frequently associated with clinical mastitis; in one study CNS spp. were isolated from 11% of mammary glands with a SCC >199 x 10^3 cells/ml and from 4.5% of clinical cases (Breen *et al.*, 2009).

Clinical mastitis cases in suckler ewes are most commonly associated with *S. aureus* or *M. haemolytica*. In a Dutch study of clinical mastitis, Koop *et al.*, (2010) isolated *M. haemolytica* and *S. aureus* from 48% and 39% of 31 mastitic milk samples respectively. In an observational study of clinical mastitis of 12, 000 ewes in 32 suckler flocks in Southern Ireland, 46% of isolates from clinical cases were of *S. aureus*, 21% *M. haemolytica* and 16% *Streptococcus* spp. (Onnasch, 2002). Similar proportions of species of causative bacteria were reported in a Norwegian study of clinical mastitis of suckler ewes: *S. aureus* was also the most predominant isolate and *E. coli* was also commonly observed, with *S. aureus* isolated from 65.3% of samples from clinically affected udder halves, enterobacteria (mainly *E. coli*) from 7.3%, *Streptococci* from 4.6%, CNS from 3% and *M. haemolytica* from 1.8% (Mørk *et al.*, 2007). Onnasch *et al.* (2002) observed that *S. aureus* associated acute clinical mastitis cases peaked in weeks 5-7 in lactation. In contrast to other pathogenic bacteria species that are associated with acute clinical cases, *S. aureus* is also commonly associated with chronic clinical mastitis (Morogna *et al.*, 2010) and

subclinical mastitis (Bergonier *et al.*, 2003) and it has been suggested that some ewes are persistently sub-clinically infected with *S. aureus* in one or both glands some of which proceed to develop acute or chronic clinical mastitis. In a cross-sectional Norwegian study of clinical mastitis in suckler sheep, 40% of 471 ewes with unilateral clinical mastitis had a subclinical *S. aureus* infection in the contralateral gland compared to only 14% of ewes that had unilateral clinical mastitis caused by other pathogens (Mørk *et al.*, 2007). *S. aureus* has also been isolated from the skin and skin lesions of the udder (Scott *et al.*, 1997).

Some strains of *M. haemolytica* are more pathogenic than others and are associated with severe acute and gangrenous mastitis (Watkins *et al.*, 1992). Like *S. aureus, M. haemolytica* has also been isolated from the skin of the udder but, in contrast to *S. aureus*, this is during lactation only (Scott and Jones, 1998; Mavrogianni *et al.*, 2007). Isolation of the bacterium from the oropharynx of lambs after lambing but not from the udder skin of pregnant ewes on the same farm suggests that lambs transmit infection to the udder skin (Scott *et al.*, 1998). Mavrogianni *et al.*, (2007) recovered identical *M. haemolytica* isolates between ewes and their lambs.

Escherichia coli is another common causative organism of clinical mastitis in dairy ewes (Lafi *et al.*, 1998) but has been more frequently reported in cows. Acute, per-acute or toxic mastitis cases associated with enteric and environmental Gram negative bacteria, particularly *E. coli*, have been well documented in dairy cows (Green *et al.* 1996) and also reported in suckler cows, particularly in the early stage of lactation (Menzies, 2000). Intramammary infections with *E. coli* have been described as transient or persistent (Döpfer *et al.*, 1999) with persistent infections associated with intracellular survival (White *et al.*, 2009). In dairy cows with low

SCC, severe mastitis cases in early lactation have been frequently associated with Gram negative pathogens, such as *E. coli,* from a contaminated environment (Barkema *et al.*, 1998).

Streptococcus species are of importance in dairy cow clinical mastitis but in sheep are commonly associated with subclinical mastitis (Lafi *et al.,* 1998). Watkins *et al.* (1991) frequently observed *Streptococcus* spp. as pure and mixed infection in subclinically infected suckler ewe mammary glands. When speciated, *Strep. uberis, Strep. faecalis, Strep. bovis and Strep. faecium* (Watkins *et al.*, 1991) and *Strep. dysgalactiae* (Mørk *et al.*, 2007) were the most common *Streptococcus* spp. isolates from milk or udder secretions of suckler ewes and *Strep. agalactiae* has additionally been described in dairy ewes with IMI (Lafi *et al.*, 1998; Linage *et al.*, 2008).

Corynebacteria spp. have infrequently been associated with both subclinical and clinical cases of mastitis in ewes (Lafi *et al.*, 1998) and are considered minor pathogens in IMIs of dairy cows (Sargeant *et al.* 2001). In a study by Breen *et al.* (2009) the isolation rates of *Corynebacteria* spp. from dairy cow mammary glands with a SCC of >199 x 10^3 cells/ml was 10% and accounted for 2.3% of clinical mastitis cases. In a survey of dairy cows with clinical mastitis and high SCC on 97 dairy farms, a similar proportion of quarters (10%) were positive for *Corynebacteria* spp. (Bradley *et al.*, 2007). Watkins *et al.* (1991) isolated *Corynebacteria* spp. from 1% of subclinically infected udder halves of suckler ewes.

Bacillus spp. and *Proteus* spp. were considered by Sargeant *et al.* (2001) to be major mastitis pathogens in cows but have been little associated with udder disease of suckler sheep. Other bacterial pathogens reported in sheep albeit with low frequency are *Arcanobacter pyogenes*, *Clostridium* spp., *Mycoplasma* spp., *Listeria*

monocytogenes and *Pseudomonas aeruginosa* (Menzies, 2000; Bergonier *et al.*, 2003).

Pathogen presence and dynamics are different in the dry and lactation periods (Green, 2003). The persistence of infections through the dry period has been little studied in suckler ewes. In a study of the effect of dry-off treatment in dairy ewes *Streptococcus* spp. associated IMIs, which were detected in about 10% of mammary glands at drying off, persisted in all mammary glands that did not receive dry-off treatment and around half of those that did receive treatment (Chaffer *et al.*, 2003). Pantoja *et al.* (2009) demonstrated that most IMIs in dairy cow quarters at dry-off did not persist through the dry period to calving. In dairy cows, although CNS bacteria are the most common species associated with pre and post dry period IMI, new IMIs during the dry period have been most commonly associated with enterobacteria, the majority of which were *E. coli* (Bradley and Green, 2000). *Corynebacteria* spp. are commonly observed in dry period infections: In one study, *Corynebacteria* spp. were isolated from 36% of lacteal secretion samples collected from dairy cow mammary glands during the dry period and were the most prevalent species isolated although most of these infections did not persist into the lactation period (Green *et al.*, 2002).

1.4 Level of inflammatory response with bacterial species

Level of SCC elevation (Gonzalo *et al.*, 2002; Pantoja *et al.* 2009), or the presence of clinical signs (Lafi *et al.*, 1998), define the bacterial species as a minor or major intramammary pathogen. High mammary gland SCC in dairy ewes associated with bacterial IMI have been observed even in the absence of other clinical signs (Leitner *et al.*, 2001) and some bacterial species provoke a greater inflammatory response than others (Arsenault *et al.*, 2008). Highest SCCs in ewes have been associated with

Mannheimia haemolytica, *Streptococcus agalactiae* and *S. aureus* (Ariznabarreta *et al.*, 2002). In dairy sheep *Mannheimia haemolytica* and *Streptococcus agalactiae* IMIs have been associated with cell counts above 7 x 10^6 cells/ml and *S. aureus* infections associated with cell counts above 3 x 10^6 cells/ml (Gonzalo *et al.*, 2002; Albenzio *et al.*, 2002). Both of these species are considered major pathogens. Minor pathogens are those associated with lower SCC counts and no signs of clinical mastitis. Lower counts of 1 x 10^5 cells/ml have been observed in association with *Corynebacterium* and *Micrococcus* spp. and Novobiocin resistant strains of CNS spp. (*Staphylococcus epidermidis, Staphylococcus intermedius*) all of which are considered minor pathogens (Ariznabaretta *et al.*, 2002). However, although CNS species in general have been considered minor pathogens, they have also been associated with high mammary gland SCC in dairy sheep (Gonzalo *et al.*, 2002; Pengov, 2001). Leitner and colleagues (2004) demonstrated that dairy ewe mammary glands subclinically infected with CNS spp. had SCCs of the order 1 x 10^6 cells/ml which was significantly higher than those without IMI. In dairy cows, low SCC is commonly associated with Gram negative IMI. A study in the Netherlands showed that, whilst *S. aureus* was cultured most often in dairy cow herds with a high bulk milk SCC, clinical mastitis cases from which Gram negative organisms such as *E. coli, Klebsiella* spp. *and Pseudomonas* spp. were isolated were associated with a low SCC (Barkema *et al.*, 1998). Conversely, in dairy ewes, Albenzio *et al.* (2002) found SCC to be higher with environmental pathogen IMI (including clinical mastitis associated with *E. coli* and *Pseudomonas* infection) than for ewes with CNS IMI or infection with contagious pathogens, including *S. aureus*.

Whilst species of pathogen is of importance, an association of SCC with bacterial load has also been demonstrated. In a cross-sectional data set from milk samples

from 50 udder halves from suckler ewes from one farm, with a mean SCC of 1.2 x 10^6 cells/ml, lower SCCs were associated with <100cfu/ml of bacteria in udder half milk samples (Smith *et al.*, 2011). Whether pathogens are considered to be major or minor, many species have been associated with both subclinical and clinical disease of a range of severity and type. This suggests that not only are bacterial species and bacterial load important in determining severity of disease but that there are other factors associated with level of inflammatory reaction with IMI.

1.5 Use of somatic cell counts to understand risk of clinical disease

Variability of SCC with different infectious causes, together with differences between studies of how infection and cut-off levels of SCC as an indicator of IMI or subclinical mastitis are defined, necessitate a cautious approach in the interpretation of findings from dairy ewe studies. Care must also been taken in the extrapolation of knowledge of udder disease directly from research in dairy cows to those of suckler sheep. Somatic cell counts of suckler sheep are higher than those of dairy cows and although this may be partially attributed to physiological and management differences, it is also likely that they have different infection levels as a result of different exposures, stressors and pathogens. Sargeant *et al.* (2001) concluded that quantitative measurement of SCC provides scope for investigating linear relationships between SCC and other variables. In order to usefully employ somatic cell counting as such a tool it is crucial to account for physiological or other factors associated with a higher or lower SCC and identify which of these are risk factors. For example, the concentration of somatic cells in milk is related to the volume of milk produced (Emanuelsson and Funke, 1991; Green *et al.*, 2006) which is a physiological factor that is associated with time in lactation. By identifying the similarities and differences between the udders of ruminants used for dairy

production and ruminants rearing offspring for meat production, we improve not only the understanding of infection patterns in the udders of suckler ewes, but the understanding of udder disease *per se*. The SCC of milk provides a continuous measure which researchers may also use to further understand the impacts of udder disease. For example, the effect of IMI on production may be assessed by investigating the association of SCC with milk yield of dairy sheep, or suboptimal lamb growth of suckling lambs in meat sheep (Fthenakis *et al.*, 1990).

The longitudinal observation of SCC at the mammary gland level informs how IMI and levels of inflammation change over time and can be used to identify points at which there is a greater risk of disease, thus contributing knowledge on how efforts may be focussed in order to prevent subclinical and clinical mastitis. Numerous studies, the majority of which are of dairy cows, have assessed the relationships between the levels and patterns of SCC with risk and severity of clinical mastitis (Barkema *et al.*, 1998; Green *et al.*, 2004.; Green *et al.*, 2006; Peeler *et al.*, 2002; Beaudeau *et al.*, 2001). In dairy sheep it has been suggested that, in mammary glands infected with CNS infection, a SCC of 400 x 10^3 cells/ml could serve as an early warning of a subclinical mastitis case emerging and 600 x 10^3 cells/ml serve as a threshold for a case of subclinical mastitis (Burriel *et al.*, 1997). Madouasse *et al.* (2012) demonstrated that dairy cows with high SCC at drying off, defined by a cow SCC >200 x 10^3 cells/ml, were more likely to have high SCC, indicating an IMI, at the beginning of the next lactation. Dairy heifers with high SCC earlier in lactation had an increased risk of clinical mastitis (Rupp and Boichard, 2000) whilst a higher incidence rate of clinical mastitis was demonstrated in dairy cows with a SCC >200 x 10^3 cells/ml (Green *et al.* 2007). However, clinical cases are not always associated with high mammary gland SCC. Increased risk of clinical mastitis with low

mammary gland SCC has also been demonstrated by Suriyasathaporn *et al.* (2000) and by Beaudeau *et al.* (2002) who described an increased risk of clinical mastitis in dairy herds which had a higher proportion of cows with SCC <250 x 10^3 cells/ml. *Escherichia coli* mastitis has been associated with low bulk milk SCC (Green *et al.*, 1996; Barkema *et al.*, 1998; Tadich *et al.*, 1998;) and low SCC has been observed in the initial stages of some acute Gram negative infections of dairy cows in early lactation (Green *et al.*, 2004). Severe early infections in dairy cows with low SCC have been demonstrated to be principally caused by Gram negative pathogens such as *E. coli* from a contaminated environment (Barkema *et al.*, 1998). This is thought to be because these largely enteric and environmental contaminants are able to cause clinical disease before an adequate immune response may be mounted (Barkema *et al.*, 1998; Schukken *et al.*, 1991). At the herd level, *Strep uberis* and *E. coli* accounted for approximately 50% of clinical cases where there was a low annual bulk milk SCC of <150 x10^3 cells/ml (Peeler *et al.*, 2003). Green *et al.* (2004) demonstrated that an increased geometric mean lactation SCC from individual quarters was associated with a decreased risk of clinical mastitis caused by *Escherichia coli* infection (Green *et al.*, 2004).

Milk SCC associated with different levels of risk of development of clinical mastitis appears to vary according to time into lactation. In one dairy cow study, a very low gland SCC of < 21 x 10^3 cells/ml was associated with an increased risk of mastitis and more severe mastitis in the following month, particularly early in lactation, when compared to higher gland SCC (Peeler *et al.*, 2003). Green *et al.* (2003) demonstrated that dairy cow mammary glands with an SCC >200 x10^3 cells/ml had an increased risk of clinical mastitis in the subsequent month. Between these levels was low/intermediate SCC range of 21 x 10^3 cells/ml - 100 x 10^3 cells/ml) that was

associated with a decreased risk of clinical mastitis and for this reason was regarded as a "protective" range. However, this range was dependent on time in lactation with lowest risk ranges of 41 $x10^3$ cells/ml -100 $x10^3$ cells/ml, 81 x 10^3 cells/ml -150 $x10^3$ cells/ml and 61 $x10^3$ cells/ml -150 $x10^3$ cells/ml in the first month, 1-2 months and 2-3 months in lactation respectively. Quarters with very low SCC (1 x 10^3 cells/ml -5 x 10^3 cells/ml) had an increased risk of coliform mastitis in the following month (Peeler *et al.*, 2002). Altogether these findings suggest that, in dairy cows at least, a low/intermediate rather than a very low mammary gland SCC is indicative of longer term stability of udder health (Green, *et al.*, 2004).

In dairy cows, the risk of clinical mastitis may also be predicted based on dynamics (Barkema *et al.*, 1998) or change in level of milk SCC (Peeler *et al.*, 2002; Green *et al.*, 2004). Green *et al.*, (2004) demonstrated that an increased maximum cow SCC and standard deviation of SCC during lactation were better predictors of clinical mastitis than geometric mean and variation of cow SCC. However, in interpreting SCC it is important to consider that milk production volume and SCC are not independent and in dairy cows temporal SCC variation has, at least in part, to do with the dilution effect as yield changes with time (Green *et al.*, 2006). As milk volume increases, so does the dilution of somatic cells in each millilitre of milk. Thus the number of somatic cells per ml could be expected to decrease during peak lactation. However Green *et al.* (2006) identified a circular relationship between infection, SCC and milk production. Whilst infection may lead to an increase in SCC it may also result in a decrease in milk production which causes less dilution of somatic cells per millilitre and thus the SCC becomes higher.

1.6 Risk factors for intramammary infection

When using SCC as an indicator of IMI, other variables associated with higher or lower SCC need to be considered. Variables may be considered as physiological, affecting milk yield and somatic cell dilution or as risk factors for IMI, although these are unlikely to be independent. There is a substantial body of evidence, from dairy cow research, of temporal variation of SCC over lactation (Green, 2003; De Haas *et al.*, 2002). A study of gland (quarter) SCC on three UK bovine dairy farms with low bulk milk SCC (<150 $x10^3$ cells/ml) showed a pattern of fluctuation of SCC occurring in quarters with time post lactation. Quarter SCC was highest in the month following lactation, lowest in the second month following lactation and steadily increased again thereafter. Quarters with a SCC range of 41 x 10^3 cells/ml – 150 x 10^3 cells/ml were least likely to fluctuate (Green, *et al.* 2004). Cell counts in this range indicate an absence of infection whereas instability of cell counts below this range suggests insufficient somatic cells to provide the aforementioned protective effect, and those above this range reflect infection and thus more fluctuation. Evidence of temporal variation also exists for dairy sheep; in a study of bulk milk from five Comisana ewe flocks in southern Italy SCC was higher towards the end of lactation than in early or mid lactation (Sevi *et al.*, 2004).

In contrast to dairy cow research, there have been relatively few studies to assess factors associated with SCC in suckler ewes. Where performed, suckler ewe SCC has been shown to be associated with stage of lactation (Hariharan *et al.*, 2004), age or parity (Hariharan *et al.*, 2004; Watkins *et al.*, 1991; Lafi *et al.*, 2006), litter size (Gonzalo *et al.*, 2002; Lafi *et al.*, 2006) and teat damage (Mavrogianni *et al.*, 2007). Risk factors for subclinical and clinical mastitis have also been relatively poorly studied in suckler ewes whilst there have been several studies investigating risk

factors for clinical mastitis in dairy cows (Green *et al.*, 2007; Peeler *et al.*, 2000; Elbers *et al.*, 1998).

There is substantial evidence that SCC and IMI increases with increasing parity of dairy and suckler ruminants. In dairy cows increased parity has been associated with increased gland SCC (Green *et al.*, 2001), a SCC of >199 x 10^3 during lactation (Breen *et al.*, 2009) and a SCC of >200 x 10^3 cells/ml at the beginning of the subsequent lactation (Madouasse *et al.*, 2012), where such levels are indicative of IMI. In a study of ewes of mixed production type, multiparous ewes had a significantly higher mean lnSCC than primiparous ewes (Lafi *et al.*, 2006). Conversely, in an analysis of dairy ewe records from a seven-year period from one University flock in the United States, SCC was highest at first lactation and decreased with successive lactations thereafter (Paape *et al.*, 2007) although this could be have been confounded by a lower milk yield in primiparous ewes. The positive association of SCC with increased parity may reflect a higher prevalence of chronic subclinical IMI from exposure to bacterial invasion over a greater number of lactations. Increasing parity was associated with an increased risk of subclinical mastitis in Swiss organic dairy herds (Busato *et al.*, 2000) and an increased incidence rate of clinical mastitis in dairy cows (Green *et al.* 2007). An increased risk of IMI with increased parity in ewes has been demonstrated in dairy ewes (Lafi *et al.*, 2006; Beheshsti *et al.*, 2010) and, in suckler ewes, the prevalence of subclinical mastitis increased with age of ewe (Watkins *et al.*, 1991; Beheshti *et al.*, 2010; Arsenault *et al.*, 2008).

Body condition score is also a risk factor for IMI and mastitis. Suckler ewes with a BCS <2.5 had an increased risk of subclinical mastitis (Arsenault *et al.*, 2008) whilst

low or high body condition score (BCS) of dairy cows was associated with an SCC of >199 x 10^3 (Breen *et al.*, 2009). However, Busato *et al.* (2000) did not find a difference in BCS of Swiss organic dairy cows with subclinical mastitis in comparison to those without. Dairy cows within herds with a high herd incidence of clinical mastitis were in higher BCS before calving and in early lactation than those in herds with a low incidence, although feeding strategies of roughage and concentrates were also of importance (Valde *et al.*, 2007).

Analysis of milk recording data from 2000 farms in England and Wales demonstrated that cows that recorded higher yields before drying off had a SCC of >200 x 10^3 cells/ml at the beginning of the subsequent lactation (Madouasse *et al.*, 2012). Jones and Jones (1986) observed that higher milk yielding cows had a higher risk of developing *E. coli* clinical mastitis. In suckler ewes, the number of lambs being reared may be regarded as a proxy for milk yield. However, transfer of bacteria to the teat via lambs' mouths may be a confounding factor in studies of suckler ewes which, additionally, do not always differentiate between the number of lambs born to a ewe and the number of lambs being reared. Litter size has been variably associated with IMI in suckler ewes. In a cross-sectional study of 46 Jordanian Awassi flocks, comprised of ewes of mixed production type that nursed until lambs were 60-90 days old before being milked for dairy production, ewes nursing twin lambs had a significantly higher mean lnSCC than ewes with single lambs. In suckler ewes, the risk of subclinical mastitis and clinical mastitis was increased in ewes rearing triplets (Arsenault *et al.*, 2008) but not twins, when compared to those rearing singles. Larsgard *et al.* (1993) observed an increased incidence of clinical mastitis in suckler ewes with increasing litter size at birth

Higher demand for milk from hungry lambs may be considered to predispose towards teat lesions. However, the definition of what constitutes a teat lesion or teat damage varies considerably (Mavrogianni and Fthenakis, 2007) thus different types of teat damage may be differently associated with SCC and IMI. Cooper (2011, Master's thesis) performed a comprehensive longitudinal study of naturally occurring external teat lesions in a suckler flock and demonstrated that teat lesions can be characterised into traumatic and non-traumatic types based on patterns of occurrence, and that ewes in poor body condition are at higher risk of developing teat lesions in the subsequent lactation. In a longitudinal observational field study of nursing ewes in 7 flocks in Southern England conducted by Watkins *et al.* (1991), no significant association was found between the presence of naturally occurring teat lesions and subclinical mastitis. In dairy cows, lower gland SCC was associated with hypercallosity of the teat tip (Breen *et al.*, 2009). Fragkou *et al.* (2007) reported that ewes with experimentally chapped teats were more susceptible to developing subclinical or clinical mastitis when experimentally challenged with *Mannheimia haemolytica*. Mavrogianni *et al.* (2006) demonstrated that teat mucosal damage was a risk factor in determining whether ewes exposed to challenge with *M. haemolytica* developed clinical mastitis.

The association of udder conformation with SCC has also been little investigated. Udder conformation traits were described by Boettcher *et al.* (1997) as a significant factor for dairy cow survival. Casu *et al.* (2010) demonstrated that, in dairy ewes, the risk of clinical mastitis or high SCC, as determined by at least 2 daily recordings of SCC > 1,000 x 10^3cells/ml over lactation, increased as cistern height increased and the degree of udder suspension and udder depth decreased. Larsgard *et al.* (1993) reported that the risk of clinical mastitis in dairy sheep increased with poor udder

conformation. Heritabilities of SCC and genetic correlations with udder traits of primiparous dairy ewes were studied by Casu *et al.* (2010) who proposed that udder depth, teat placement and degree of udder suspension should all be appraised to reduce clinical mastitis and improve overall flock health, although another study stated that stage of lactation and parity influence the assessment of udder conformation (de la Fuente., 1996). Udder confirmation is likely to vary with breed and production type and the risk of clinical mastitis has indeed been shown to differ with sheep breed (Larsgard *et al.*, 1993) and other genetic factors. Heritability of mastitis in Danish Holstein dairy cows was described as low (0.025) by Lund *et al.* (1994) whilst that for SCC was higher (0.18). Fernandez *et al.* (1997) concluded that heritabilities for udder conformation traits in dairy ewes were similar to those in dairy cows. However, there are likely to be many reasons for breed susceptibility and an appraisal of the genetics conducted by Conington *et al.* (2008) stated that more research was needed to improve knowledge of genetics of mastitis in suckler ewes.

An increased incidence rate of clinical mastitis in dairy cows has been associated a variety of herd hygiene management practices, for example, the use of dry cow antibiotic treatment across the whole herd, rather than targeting individual cows (Green *et al.*, 2007). However, the management practices of dairy cows are very different to those of suckler ewes which are exposed to different hazards. Environmental factors are also of importance. The risk of clinical mastitis has been shown to increase with grazing environment (mountain versus cultivated pasture) in suckler ewes (Larsgard *et al.*, 1993) and dairy cows, which, during housing periods, had a higher prevalence of subclinical mastitis when housed in alpine barns than lowland barns (Busato *et al.*, 2000).

Risk of IMI is also associated with stage of lactation although, as already discussed, the association of milk yield with SCC must be taken into consideration. Breen *et al.* (2009) reported that, in dairy cows, an SCC of >199 x 10^3 was associated with increasing month of lactation. An increased risk of subclinical mastitis was also demonstrated with increasing stage of lactation in a study of Swiss organic dairy herds (Busato *et al.*, 2000). Risk of clinical mastitis is also associated with stage of lactation. In suckler ewes, a peak of clinical cases is commonly observed in the first week post-partum and the majority of these in the first couple of days (Mørk *et al.*, 2007). These may be new IMIs acquired in early lactation or those arising from the previous dry period. In dairy cows clinical cases associated with dry period infections were most likely to occur earlier on in lactation in mammary glands from which the same bacteria was isolated more than once in the late lactation and early lactation period (Green *et al.*, 2002). Later peaks of suckler ewe clinical mastitis cases were also observed in weeks 3 to 4 of lactation in a Norwegian study (Mørk *et al.*, 2007) and at weeks 4 and 7 of lactation in an Irish study (Onnasch, 2000, Master's thesis). These later periods roughly coincide with periods of peak lactation but also rapid lamb growth and incisor development which may contribute to teat lesions although the role of teat damage in udder infection and mastitis needs further work (Cooper, 2011, Master's thesis).

1.7 The impact of intramammary infection in suckler ewes

Impact of intramammary infection on production

Subclinical mastitis decreases milk production of dairy sheep (Saratsis *et al.*, 1999; Gonzalo *et al.*, 2002). In suckler ewes, suboptimal lamb growth may therefore be partly attributed by the negative effect of IMI on milk production; a decrease of 20-37% in milk production of meat sheep was projected to be associated with a 4kg

difference in lamb weight at weaning (Menzies, 2000). In suckler sheep, several studies have demonstrated lower weights of lambs reared by ewes with subclinical or clinical mastitis. In a controlled trial investigating the effect of subclinical mastitis on lamb growth, lambs reared by ewes with experimentally induced subclinical IMI with *Staphylococcus simulans* had significantly lower growth rates and weighed up to 30% less at 52 days of age than lambs reared by unchallenged ewes (Fthenakis and Jones, 1990). Similarly, a longitudinal study of 169 lambs reared by 115 suckler ewes demonstrated that lambs reared by ewes with no bacteria isolated from milk collected on five occasions between 1 and 50 days in lactation weighed 3.5kg more at 50 days of age than those reared by ewes where bacteria was isolated (Moroni *et al.*, 2007). Whilst both of these studies assessed the association of lamb growth with the presence of bacteria in the milk, the effect of level of udder disease on lamb weight is difficult to adequately assess without using a continuous measure of udder disease, such as the somatic cell count of milk, rather than presence of absence of bacteria. The semiquantitative CMT method was adopted in a study of 261 ewes on 30 farms by Arsenault *et al.* (2008) where weaning weight of lambs reared by ewes with a positive CMT score was significantly lower compared with those reared by ewes with negative CMT. Keisler *et al.* (1992) demonstrated that this effect was not observed when lambs were offered supplementary feed in the first 8 weeks of age. In one study, lambs reared by ewes with clinical mastitis in the lactation weighed 2kg less at 45 days of age and 4kg less at 145 days of age than those reared by ewes with no clinical signs of udder disease (Larsgard and Vaabenoe, 1993). Increased lamb mortality was significantly associated with clinical mastitis in suckler ewes (Arsenault *et al.*, 2008). In general, studies investigating the effect of mastitis on

lamb weight are relatively low in number and have not fully investigated the effect of, or adjusted for, other factors associated with lamb weight or udder disease.

Financial impact of intramammary infection

Financial losses due to mastitis in suckler flocks arise not just because of decreased production, but also because of costs of disease treatment and ewe replacements. Ewe losses may result from the affected ewe dying or being culled early with associated costs of ewe replacements (Conington *et al.*, 2008). In an Irish study of commercial non-dairy flocks, 26% of cull ewes were culled because they had chronic mastitis and the prevalence of chronic mastitis in a survey of cull ewes was between 2.8 and 3.3 % (Onnasch, 2000, Master's thesis). However, it appears that robust estimates for the financial cost of meat sheep mastitis in the UK are not available.

1.8 Treatment and management of mastitis

Medical treatment costs of affected ewes include the price of pharmaceuticals and time to administer treatment. Treatment of clinical mastitis cases in sheep is usually reactive and aimed at salvage of the ewe and maintaining a functional udder for rearing the lamb. The main approach for treatment is an antibiotic administered either intramammarily or parenterally, with or without an antinflammatory. The effectiveness of treatment and management strategies vary according to pathogen involved, severity of disease and speed of treatment, thus success may be greater in dairy animals as signs of disease may be detected earlier and when disease is subclinical.

In dairy animals, particularly dairy cows, there is a focus on the prevention of infections in early lactation by using antibiotics to clear existing infection at the beginning of the dry period by the use of intramammary antibiotic treatment, known

as dry cow therapy (DCT), with or without the use of a teat sealant to prevent ascending infection (Huxley *et al.*, 2002). Dry cow therapy is frequently used in dairy herds to reduce the risk of subsequent mastitis. There are currently no licensed products for use in sheep although bovine therapy may be administered under the cascade system for veterinary medicinal treatment. Whilst such prophylactic treatments are rarely administered in sheep, when used, dry period antimicrobial prophylaxis appears to be mainly practised in dairy flocks rather than suckler flocks, often sharing one intra-mammary tube between the two teats of the same ewe. In dairy cows, a broad spectrum DCT preparation was previously demonstrated to be effective in removing Gram negative and Gram positive pathogens at drying off and was effective in the prevention of *E. coli* mastitis in the first 100 days of lactation (Bradley *et al.*, 2001). Green *et al.* (2007) reported that, whilst individual cow treatment was associated with a decreased risk of clinical mastitis in the subsequent lactation, whole herd treatment with DCT was associated with an increased risk. Similarly, in a New Zealand study, the use of DCT in bovine dairy herds with a low prevalence of mastitis resulted in an increased herd prevalence of mastitis (Cagienard, 1983) and Dutch dairy herds with a long history of dry cow therapy treatment had a higher incidence rate of clinical mastitis (Barkema *et al.*, 1995).

In dairy ewes, Linage *et al.* (2008) demonstrated a significant decrease in SCC at subsequent lambing following administration with a broad spectrum product at dry-off. In a study of antibiotic treatment at dry off of dairy sheep a beneficial effect on clinical mastitis was observed in early lactation only (Chaffer *et al.*, 2003). The benefits of administration of antimicrobial dry off treatment may depend on the spectrum of activity of the preparation.

1.9 Rationale for further research

Knowledge gaps

Knowledge of longitudinal patterns, risk factors and effects of IMI in suckler ewes is limited. In order to use SCC as a research tool to investigate IMI of suckler ewes, a greater understanding of the patterns of SCC and risk factors for higher or lower SCC is necessary. However, the longitudinal pattern of SCC of suckler ewes over lactation has not been well described nor have physiological factors associated with higher or lower SCCs over lactation been sufficiently studied. Without this information, risk factors for IMI over lactation cannot be accurately identified.

Whilst the effect of clinical mastitis in sheep may be evident for the farmer, the effect of subclinical IMI together with udder health factors such as udder conformation and teat lesions on lamb production in the UK is unknown. Practical management approaches that farmers may take in order to prevent IMI on sheep farms have been little suggested and the potential benefits of implementing prevention strategies have been poorly quantified.

The magnitude of elevation of SCC associated with presence and load of different bacterial species is not well studied in suckler ewes. There is a need to identify which bacterial species are most associated with subclinical and clinical disease, and what levels of udder infection are typical in UK suckler ewes. The use of SCC, together with observations for trends of infection with different bacterial species through lactation, could potentially help to identify whether the risk of infection with some bacteria species is associated with stage of lactation and be used to assess the impact of bacterial infection with stage of lactation.

Relevance of existing studies

Several studies have investigated clinical mastitis (Calavas *et al.*, 1997; Kirk *et al.*, 1996; Onnasch, 2000; Onnasch *et al.*, 2002), subclinical mastitis (Fthenakis, 1994; Watkins *et al.*, 1991; McDougall *et al.*, 2002; Leitner *et al.*, 2004; Gonzalo *et al.*, 2002) and intramammary infections in ewes (Ariznabarreta *et al.*, 2002, Pengov, 2001). With the exception of one study which was conducted in the Republic of Ireland (Onnasch, 2002), and two less recent studies conducted in England (Watkins *et al.*,1991; Fthenakis, 1991), studies have been of ewes primarily used in dairy production and have been outside the British Isles. There have been no recent or comprehensive published studies of intramammary infection, subclinical mastitis and clinical mastitis in suckler ewes in the UK.

Studies in Europe may not be representative of ewes in the UK because of differences in the structure of the respective sheep industries and should be cautiously interpreted. In the UK sheep are kept primarily to produce lambs for the meat industry and ewes typically nurse their lambs for several months after which the ewes are allowed to cease lactation (Watkins *et al.*, 1991). Conversely in dairy systems which predominate in Europe, sheep are farmed for milk production and the length of time which lambs are allowed to suckle varies. Many systems are mixed and allow the lambs to suckle for up to a month or more before the lambs are weaned. Ewes are then milked for an extended period following the removal of lambs, thus maximising both lamb growth and milk production for the dairy industry (McKusick *et al.*, 2001; Kirk *et al.*, 1996). Although some European studies collect samples from a nursing period prior to weaning and some data from ewes rearing lambs exist, the nursing period is shorter and the previous exposures to pathogens of

these ewes may be different, particularly for multiparous ewes which have previously been milked.

Hypotheses

There were several hypotheses in the studies in this thesis. The first was that SCC exhibits a pattern that can be described by days in lactation, by body condition score of the ewe and by bacterial infection. The second was that IMI of suckler ewes has an adverse effect on lamb growth and that there is an association between teat lesions and udder conformation and lamb weight. The third was that SCC can be described by the presence of teat lesions, udder conformation and lamb weight. The fourth was that the use of dry cow therapy (DCT) in suckler sheep has a beneficial effect on lamb growth by removing subclinical infection. The fifth was that the use of DCT in suckler ewes is beneficial in reducing incidence of clinical mastitis of suckler ewes in the next lactation.

1.10 The aims of the studies

The aim of the first study was to investigate longitudinal trends in SCC in suckler ewes by recording SCC and bacterial species present through lactation.

The aim of the second study was to identify risk factors associated with higher SCC and the impact of SCC on lamb growth to provide further understanding of how subclinical infection may affect productivity.

In the third study, two intervention trials were conducted to investigate the effect of dry off treatment on flock levels of subclinical and clinical mastitis respectively. One aim was to assess whether the use of "dry off" antibiotic therapy at weaning reduced levels of subclinical disease on farms with a low incidence of clinical mastitis. This

was measured by recording SCC and lamb weight and investigating whether ewes which received treatment with a broad-spectrum DCT at weaning had lower SCCs and reared heavier lambs over the first 2 months of the subsequent lactation. The second aim was to assess the effect of DCT on the incidence of clinical mastitis cases in suckler ewes in the subsequent lactation on a farm with moderate to high annual incidence of clinical mastitis, to investigate whether ewes that received broad spectrum intramammary antibiotic at weaning were at significantly lower risk of developing clinical mastitis in the subsequent lactation than ewes that did not receive treatment.

2. CHAPTER 2: A STUDY TO INVESTIGATE LONGITUDINAL PATTERNS OF SOMATIC CELL COUNT IN THE FIRST TWO MONTHS OF LACTATION IN SUCKLER EWES

2.1 ABSTRACT

In order to understand how mastitis in suckler ewes may be prevented, it is necessary to improve knowledge of longitudinal patterns of intramammary infection (IMI) and how this relates to udder disease.

A cohort of 48 suckler ewes on one farm was followed for 8 weeks from lambing to weaning. Milk samples were collected at weekly intervals from each udder half of each ewe and analysed for udder half somatic cell count (HSCC) as a measure of the inflammatory status of the mammary gland. Milk samples were also collected for bacteriological screening using a one plate culture technique. The age of the ewe, the number of lambs being reared and new teat lesions were also recorded. A multilevel model was constructed to analyse the data. Udder half somatic cell count (HSCC) exhibited a pattern that was explained by days in lactation and the quadratic of days in lactation. Udder half somatic cell count was significantly ($p < 0.05$) higher in ewes older than 5 years of age. Presence of culturable Coagulase Negative *Staphylococcus* spp. and Gram negative bacteria in the milk was significantly associated with lower HSCC.

2.2 INTRODUCTION

There have been very few studies to simultaneously assess the longitudinal pattern of SCC through lactation in suckler ewes with the longitudinal pattern of bacterial types in milk of udder halves through lactation, whilst investigating and accounting for the

effect of ewe age and the presence of teat lesions. A longitudinal study by Hariharan *et al.* (2004) investigated the association of SCC with infection of the udder of 50 suckler ewes with minor and major bacterial pathogens, over 10 weeks of lactation in a Scottish flock but, although the study accounted for the effect of ewe age, it did not investigate the association with teat lesions or account for repeated measures of the data within ewes. An earlier longitudinal study by Watkins *et al.* (1991) investigated the association of bacterial infection, age of ewe and the presence of teat lesions with prevalence of subclinical mastitis of suckler ewes in seven flocks in Southern England. However, somatic cells were not directly counted in the study but instead the Whiteside test was used as an indirect indication of level of SCC. The dependent variable was subclinical mastitis rather than SCC whilst the analytical methods used left scope for improvement to further adjust for associations with explanatory variables in the analysis.

The aims of this study were to identify the patterns of SCC through lactation in the udder of nursing ewes and to identify variables which explain changes in SCC through lactation. We tested the hypotheses that there is a temporal pattern of HSCC of suckler ewes during lactation and that HSCC can also be explained by age of the ewe, the number of lambs being reared, the occurrence of teat damage and by the presence of viable bacterial species in the milk from the udder half. We also investigated whether there were patterns of infection with different bacteria with stage of lactation.

2.3 MATERIALS AND METHODS

2.3.1 Study farm and ewe selection

An 800 ewe indoor lambing and indoor rearing flock in Oxfordshire, England was convenience selected for the study. The ewes were a cross of Finnish Landrace, Poll Dorset and British Milk Sheep and lambed over a 3 week period in January 2009.

2.3.2 Calculation of sample size:

A minimum of 35 ewes were required to detect a minimum period prevalence of subclinical mastitis of 10% with 95% confidence. A sample size of 50 ewes was selected to allow for loss to follow up.

2.3.3 Collection of data

At lambing, ewes were given an individually numbered ear tag and ewe age and number of lambs in the litter were recorded. Milk samples were collected from ewes within the first 24 hours of lambing and then at 1, 2, 3, 4, 5, 6 and finally at 8 weeks after lambing, when lambs were weaned. Abnormalities in the appearance of the udder were recorded at each visit.

2.3.4 Collection of milk samples

Prior to milk collection from each udder-half, clean latex gloves were worn. Each udder half was cleaned separately with a coarse paper towel to remove soiling. The udder was palpated and abnormalities of the teat and udder were recorded. The first two strips of milk were examined in a clean paper cup which was hygienically discarded, and abnormalities of the milk were recorded. Teats were cleaned twice with 70% ethanol, concentrating on the teat tip and orifice.

From each udder-half of each ewe at each sampling event, five ml of milk was collected into a sterile universal tube containing 200µl autoclaved glycerol for

bacteriological analysis. Ten ml of milk was collected into a plain pot containing a bronopol preservative pill for SCC. Colostral milk that was very thick in consistency was not collected for somatic cell counting. Each udder half milk sample for bacteriology and SCC was given an identical number.

2.3.5 Bacteriological analysis of milk samples

Milk samples for bacteriology were put on ice after collection and transported to the University of Warwick where they were frozen at -20°C. Within 1 week of collection the samples were transported on ice to an external laboratory, Quality Milk Management Services Ltd, Somerset, UK (QMMS) where they were screened for bacterial isolates using a one-plate growth technique on (BHI) agar supplemented with 5% sterile Sheep Blood Agar (SBA). Identification was based on gross morphology by an experienced microbiologist, with the classification of each isolate as close to species level as could be determined by this method. Positive growth was defined as 3 or more colonies and, depending on the approximate number of colonies, was subjectively classified as scant, moderate or heavy growth according to the judgement of the microbiologist. Milk samples yielding more than 3 species of bacteria were defined as contaminated and not included in the analysis. Repeatability of bacterial identification by gross morphology, and the classification of samples as contaminated or not yielding growth, was assessed by aseptically splitting 39 samples at the University of Warwick and single blinding the test technique at QMMS. For each of the bacterial groups, and for samples classed as contaminated or as having no growth, test bias for the matched samples was assessed using a McNemar's chi-squared test and test agreement assessed by calculating the Kappa test statistic.

2.3.6 Somatic cell counting of milk samples

Milk samples for somatic cell counting were diluted with Phosphate Buffered Saline (PBS) up to a minimum of 20ml, to facilitate automated processing. Diluted samples were kept chilled at the University of Warwick Laboratory for a maximum of one week before being transported to QMMS for analysis. The dilutions made were recorded and results corrected accordingly. The number of somatic cells per ml was counted using an automated combined spectrometer and flow cytometer (Delta CombiScope FTIR (Delta Instruments B.V., Drachten, Netherlands)).

2.3.7 Data storage

A database was constructed in Microsoft Access 2007 into which observation date, ewe ID, body condition score, HSCC and abnormalities of the udder, teat and milk were recorded.

2.3.8 Data analysis

Descriptive analysis was performed in Stata 10 (StatCorp LP, Texas). The HSCC data were log transformed and normality visually assessed using a Q-Q plot. Strata were merged where adjacent categories had less than five observations. Difference in $\log_{10}$ HSCC for paired observations of left and right udder halves was assessed using a paired t-test. Difference between mean $\log_{10}$ HSCC for all left udder halves and mean $\log_{10}$ SCC for all right udder halves, including non-paired observations, was assessed using a non-paired t-test. Log_{10} HSCC was plotted over time by ewe age and litter size.

A multilevel model was constructed with $\log_{10}$ HSCC as the continuous outcome variable with observation, udder-half and ewe as level 1, 2 and 3 random effects (MLWin 2.11, Rasbash *et al.*, 2005).

The model took the general structure

$$y_{ijk}=\beta_0+\beta x_{ijk}+\beta x_{jk}+\beta x_k+v_k+u_{jk}+e_{ijk}$$

…..where y*ijk* was the continuous outcome variable Log_{10} SCC and βX was a series of vectors of fixed effects that vary at levels *ijk* (observation), *jk* (udder-half) and *k* (ewe), with errors *v, u* and *e.* The independent variables ewe age, days in lactation, litter size, the observation of a new teat lesion, and bacterial species were tested in the model using a manual forward stepwise selection process. Significance was set at 0.05. Variables that were significant in the univariable analysis were not retained in the final model if they became non-significant in the multivariable analysis. Where similar and highly correlated explanatory variables were tested and significant in the multivariable model, the variable that most improved the model fit to the data was retained.

2.4 RESULTS

Two ewes were lost to follow up. Forty-eight ewes were studied for the 8 week follow-up period. With the exception of one ewe with one non-productive udder half, each udder half of each ewe was sampled at every sampling event, thus there were 669 data points from 95 udder halves on 8 occasions.

2.4.1 Descriptive analysis

The majority of ewes in the study were 2 years (38%) or 3 years (25%) old. Ninety percent of study ewes were in at least their second lactation; 13% of study ewes had previously had 5 or more lactations. The majority (77%) of ewes were rearing twins. The period prevalence of teat lesions was high, with 63% of ewes and 48% of all udder halves having a teat lesion at least once point during the study (Table 2.1).

Table 2.1 Summary statistics of ewe categorical variables

Categorical variables	No. of observations	Total observations	Percentage of observations
Ewe age at lambing			
1 yr	5	48	10.42
2 yr	18	48	37.50
3 yr	12	48	25.00
4 yr	7	48	14.58
5 yr	2	48	4.17
8 yr	1	48	2.08
10 yr	3	48	6.25
Litter size			
one lamb	3	48	6.25
two lambs	37	48	77.08
three lambs	8	48	16.67
Ewe had a teat lesion on at least one teat at any point over study period			
No	18	48	37.50
Yes	30	48	62.50
Udder-half had a teat lesion at any point over study period			
No	49	95	51.58
Yes	46	95	48.42

2.4.2 Udder half somatic cell count

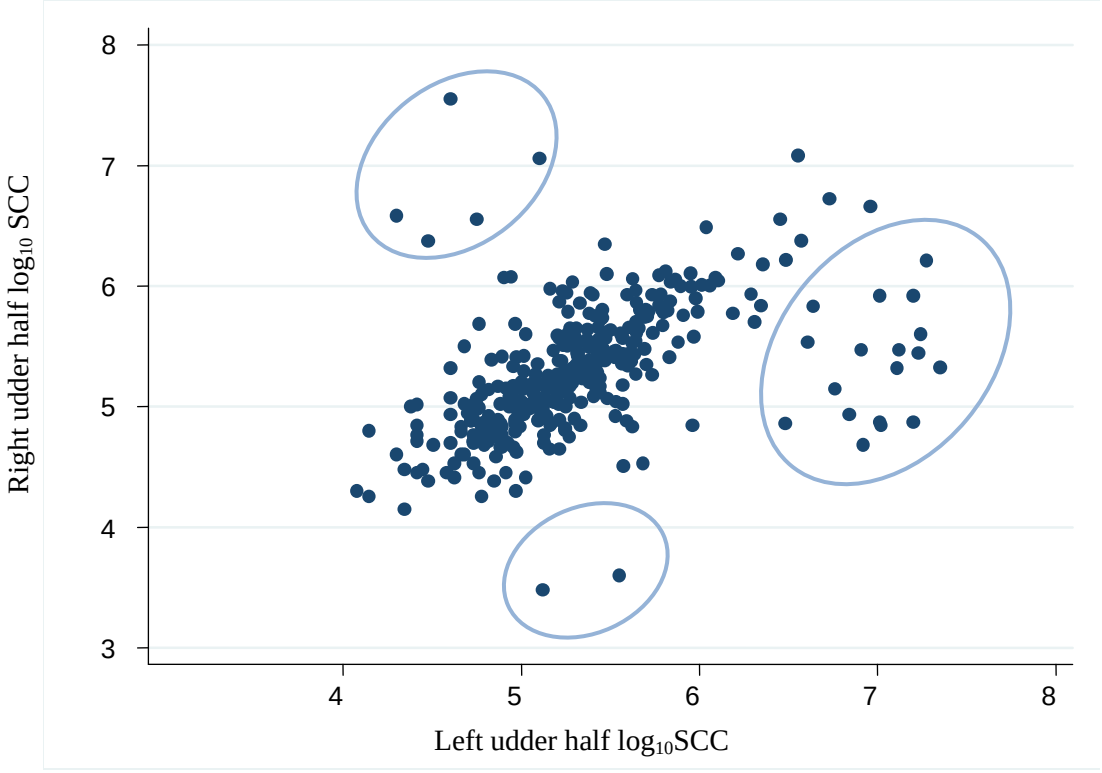
The arithmetic mean of all observations of HSCC was 725 x 10^3 cells/ml, the geometric mean 204 x 10^3 cells/ml ($\log_{10}$ HSCC of 5.31). Log_{10} HSCC provided a good fit to a Normal distribution (Figure.A.2.1). Mean HSCC was similar for left and right udder halves (Table 2.2) and there was no significant difference in $\log_{10}$ HSCC between paired observations in left and right udder halves ($p = 0.17$).

Table 2.2 Summary statistics and observations of Log_{10} HSCC

Log_{10} HSCC					
Continuous variable	**Min**	**Max**	**Mean**	**Std Dev**	**Observations**
left udder-half	4.08	7.35	5.33	0.59	341
right udder-half	3.48	7.55	5.29	0.54	328
all udder-halves	3.48	7.55	5.31	0.57	669

There was moderate significant correlation of log_{10} HSCC of left and right udder halves ($p < 1 \times 10^{-3}$, r = 0.47 between 343 paired observations). However, paired observations were less correlated at extremes of HSCC, above 6.3 log_{10} cells/ml and below 3.7 log10 cells/ml (three outlying clusters (Figure 2.1)). Thus the two halves of most udders showed greater independence at extremes of HSCC. Strong positive correlation where observations of left and right udder halves were in the ranges > 3.7 and < 6.3 log_{10} cells/ml was otherwise observed ($p < 1 \times 10^{-3}$, r = 0.71 between 312 paired observations).

Figure 2.1 Scatter plot of left and right log_{10} HSCC



There was a temporal pattern of HSCC with an overall decrease with days in lactation but with a subsequent but less pronounced rise towards the end of lactation. Mean log_{10} HSCC was significantly different ($p < 1 \times 10^{-3}$) across weeks of lactation

(Table 2.3) and decreased from lambing to a trough at around 4 weeks in lactation, and then rose again until the final observation at the point of weaning (Table 2.4 and Figure 2.2). The coefficient of variation was similar (range 0.08 to 0.12) across all weeks. In the univariable analysis of weeks in lactation within the hierarchical model, log_{10} HSCC was significantly higher at lambing than at weeks 1, 4 and 6 in lactation (Table 2.16).

Table 2.3 ANOVA of log_{10} HSCC by week of lactation

Log_{10} HSCC		No. of obs	669
		F	9.28
		Prob > F	$<1 \times 10^{-3}$
	SS	df	MS
Between weeks of lactation	21.80	8	2.73
Within weeks of lactation	193.79	661	0.29
Total	215.60	669	0.32
Bartlett's test for equal variances		$\chi^2(8) = 14.28$	Prob> = 0.075

Table 2.4 Log_{10} HSCC by week in lactation.

Log_{10} HSCC						
Wks in lactation		**95% Confidence Interval**				
	Mean	**Lower**	**Upper**	**Median**	**CV**	**n**
0	5.43	4.51	6.35	5.32	0.09	67
1	5.29	4.41	6.17	5.22	0.08	84
2	5.12	3.99	6.26	4.99	0.11	92
3	5.11	3.86	6.35	5.02	0.12	93
4	5.24	4.14	6.33	5.17	0.11	91
5	5.36	4.31	6.41	5.32	0.10	96
6	5.29	4.28	6.30	5.20	0.10	62
7	5.67	4.65	6.69	5.61	0.09	41
8	5.74	4.67	6.80	5.84	0.09	43
Over all weeks	5.31	4.20	6.42	5.25	0.11	669

Log_{10} HSCC was similar across ewes in age groups of 5 years or less, but significantly higher ewes older than 5 years of age (Table 2.16) although ewes that were older than five years old were relatively few in number and only consisted of

ewes of ages 8 and 10 years of age. Coefficient of variation was heterogenous across age groups (Table 2.5).

Figure 2.2. Box and whisker plot of log_{10} HSCC by week of lactation

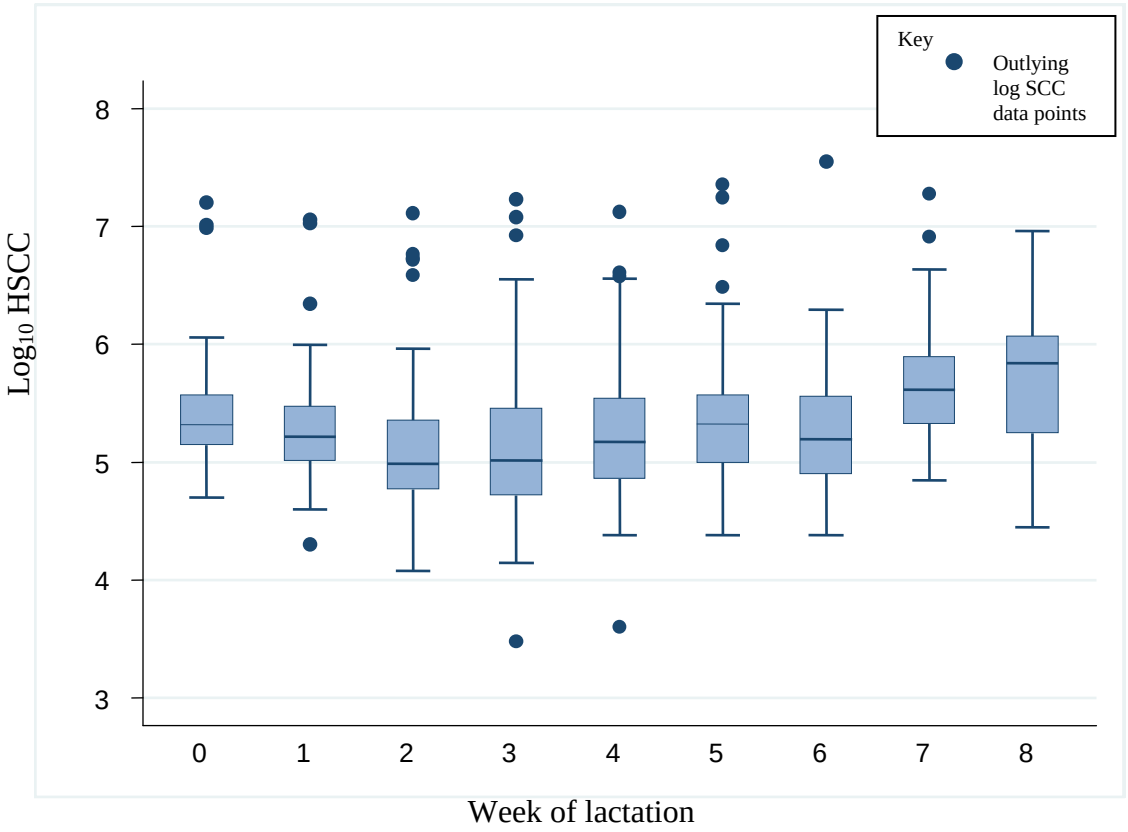


Table 2.5 Log_{10} HSCC by age of ewe

	Summary of Log_{10} HSCC					
Age	Mean	95% CIs		Median	CV	N obs
		lower	upper			
1	5.28	4.38	6.18	5.20	0.09	72
2	5.19	4.36	6.02	5.17	0.08	250
3	5.39	4.29	6.50	5.38	0.10	166
4	5.13	3.91	6.35	5.06	0.12	99
5	5.38	4.49	6.27	5.43	0.08	28
8	6.16	4.43	7.88	6.11	0.14	16
10	5.84	4.47	7.21	5.83	0.12	38
Total	5.31	4.20	6.42	5.25	0.11	669

However there were unequal variances between ewe age groups, possibly due to the low number of observations of some age groups.

The coefficient of variation was higher in ewes rearing singles and triplets as most observations were made of ewes rearing twins (Table 2.6). Although mean lower HSCC was observed in ewes rearing fewer lambs, log_{10} HSCC was not significantly different in ewes rearing singles, twins or triplets, in the univariable hierarchical model.

Table 2.6 Log_{10} HSCC by number of lambs reared

Log_{10} HSCC						
Number of lambs	95% Confidence Interval					
	mean	lower	upper	CV	median	N obs
1	5.51	4.03	7.00	0.14	5.29	42
2	5.31	4.26	6.37	0.10	5.25	531
3	5.21	4.00	6.41	0.12	5.16	96
Total	5.31	4.20	6.42	0.11	5.25	669

2.4.3 Repeatability of bacterial identification with one plate culture technique

The one culture plate technique for bacterial identification was repeatable on the single blinded assessment performed (Table A.2.1). There was no significant test bias between the two culture and identification events, except for samples classed as having no growth (McNemar's $\chi^2 = 4.00$, $p = 0.046$) (Table A.2.2), and better than expected agreement for all groups, with between fair and perfect agreement depending on group (Kappa range, 0.3 to 1.00) (Table A.2.3). Therefore there was no significant difference between the identification of bacteria from the two repeated tests.

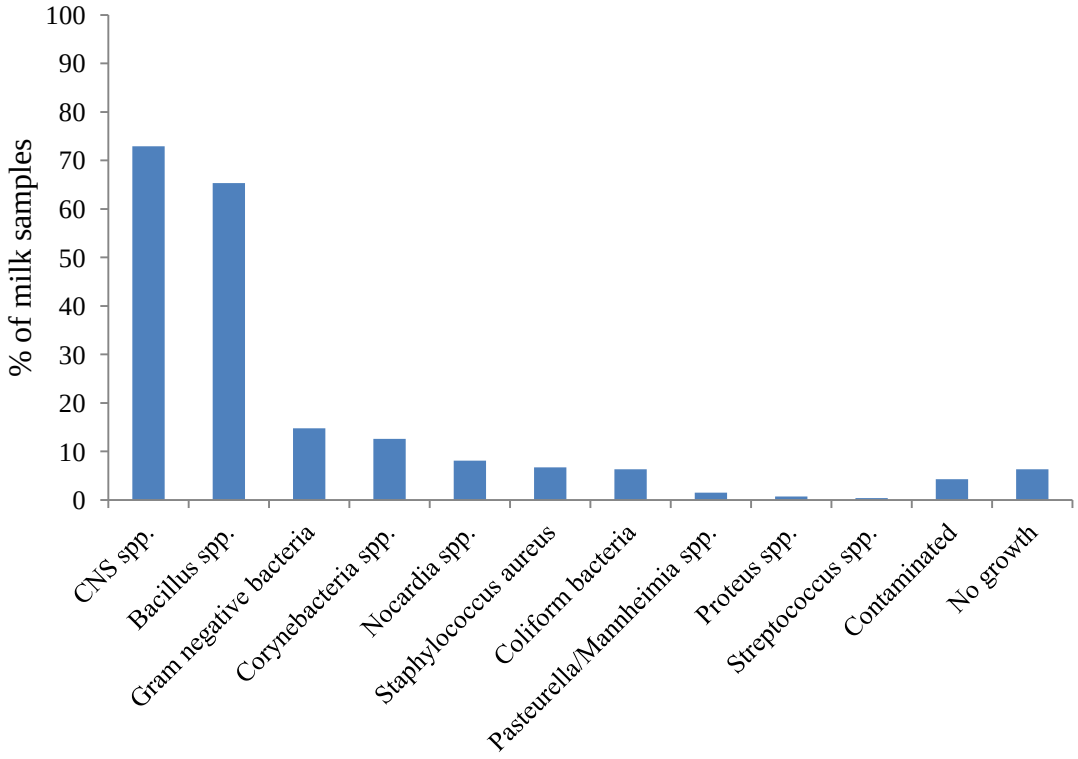
2.4.4 Bacteria cultured from milk samples

Bacteria were cultured from 627 (93.7%) of milk samples, 29 (4.3%) samples were classed as contaminated and 42 (6.3%) samples yielded no bacterial growth. Growth of two bacteria species per sample was observed more frequently (40.5%, n=271) than growth of a single species (26.4%, n=177) or three species 21.7% (n=145), indicating that most udder halves had a mixed infection. A small proportion of samples (4.3%, n=28) yielded growth of more than three species and were classed as contaminated. Only 6.3% (n=48) of samples yielded no bacterial growth at all. Whilst the proportion of samples from which growth of two species of bacteria was observed did not show any pattern with age of ewe (Figure A.2.2) or weeks of lactation, the frequency of isolation of three species of bacteria and of contaminated milk samples was higher in weeks 0 and 1 than later on in lactation. Milk samples yielding no growth or only one species of bacteria were more commonly collected from ewes in 5-8 weeks of lactation compared with earlier in lactation (Figure A.2.3). The most common isolates were Coagulase negative Staphylococcus spp. (n=488, 72.9%) and *Bacillus* spp. (n=437, 65.3%) (Table 2.7, Figure 2.3).

Table 2.7 Number and percent of samples by species/family cultured.

Bacteria	**n samples with positive growth**	**% of samples with positive growth**
Coagulase negative *Staphylococcus* spp	488	72.9
Bacillus spp.	437	65.3
Gram negative bacteria	99	14.8
Corynebacteria spp.	84	12.6
Nocardia spp.	54	8.1
Staphylococcus aureus	45	6.7
Coliform bacteria	42	6.3
Pasteurella/Mannheimia spp.	10	1.5
Proteus spp.	5	0.7
Streptococcus spp.	3	0.4
Contaminated	29	4.3
No growth	42	6.3

Figure 2.3 Percentage of positive isolates from milk samples by bacterial species

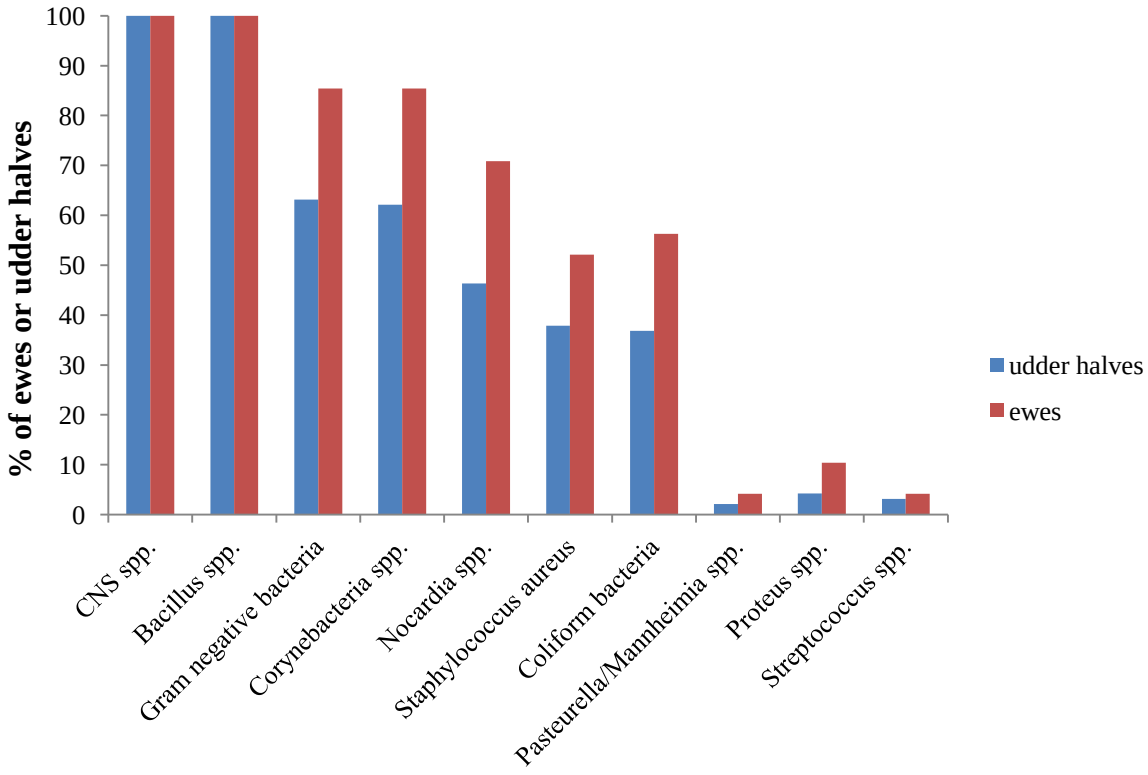


Coagulase Negative Staphylococcus spp. and *Bacillus* spp. were isolated at least once from all udder halves and all ewes (100%) over the observation period whereas *Mannhemia spp.*, *Proteus* spp. and *Streptococcus* spp. were isolated from less than 5% of udder halves and ewes (Table 2.8 , Figure 2.4).

Table 2.8 Bacteria species isolated from udder halves and ewes

Bacteria	**Udder halves (n=95)**		**Ewes (n=48)**	
	n	%	n	%
Coagulase negative Staphylococcus spp.	95	100.00	48	100.00
Bacillus spp.	95	100.00	48	100.00
Gram negative bacteria	60	63.16	41	85.42
Corynebacteria spp.	59	62.11	41	85.42
Nocardia spp.	44	46.32	34	70.83
Staphylococcus aureus	36	37.89	25	52.08
Coliform bacteria	35	36.84	27	56.25
Pasteurella/Mannheimia spp.	2	2.11	2	4.17
Proteus spp.	4	4.21	5	10.42
Streptococcus spp.	3	3.16	2	4.17

Figure 2.4 Percentage of ewes and udder-halves in which bacteria was isolated across all visits



2.4.5 Correlation of bacterial species

There were no strong correlations between bacterial species isolated (Table 2.9)

Table 2.9 Correlations of bacterial species isolated

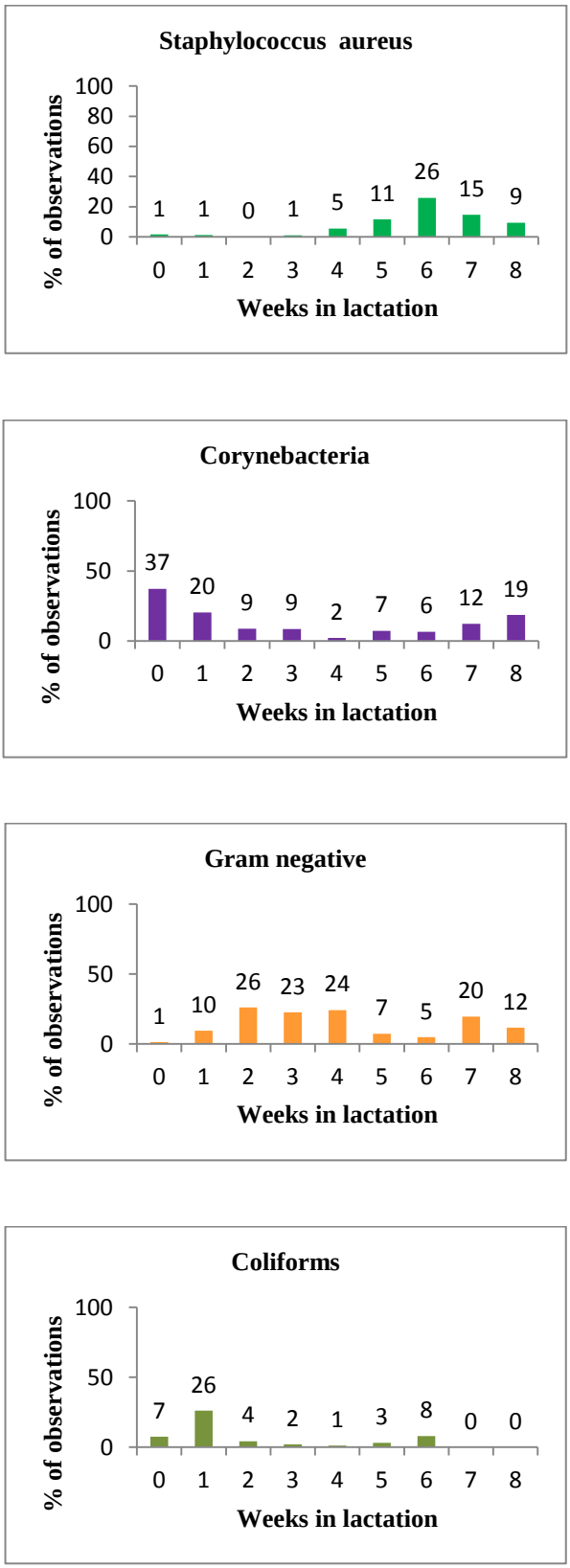
	Bacillus spp	Coliforms	Coliforms	Fungal	Gram negative spp	Nocardia	Mannheimia	Proteus	Coagulase negative spp	Staph aurues
Bacillus	1									
Coliforms	0.09*	1								
Corynebacteria	0.009	0.01	1							
Fungal	0.05	0.15*	-0.00	1						
Gram negatives	0.11*	-0.06	-0.02	-0.04	1					
Nocardia	0.111*	-0.03	0.07	-0.06	-0.06	1				
Mannheimia	-0.14*	-0.03	-0.05	-0.02	-0.05	0.01	1			
Proteus	-0.01	-0.02	-0.03	-0.08	-0.04	-0.03	-0.01	1		
CNS	0.16*	0.07	0.02	0.01	0.07	-0.02	-0.15*	-0.03	1	
Staph aureus	-0.08*	-0.05	-0.07	-0.05	-0.06	-0.06	-0.03	-0.02	-0.08*	1
Streptococcus	-0.05	-0.02	-0.03	-0.01	0.04	-0.02	-0.01	-0.01	0.04	-0.02

* indicates significance p<0.05

2.4.6 Bacterial species isolated by stage of lactation.

For some bacterial species, the frequency of isolation was associated with stage of lactation. *Staphylococcus aureus* was mainly cultured from milk samples collected in the second half of lactation, with peak prevalence in week 6 (Figure 2.5). The pattern of isolations of *Nocardia* (Figure A.2.4.) and *Corynebacteria* (Figure 2.5) were similar and were most prevalent at lambing, reduced in prevalence as lactation progressed, then increased in prevalence towards the end of lactation. Conversely, Gram negative bacteria (Figure 2.5) showed an inverse pattern to *Corynebacterium* spp. and *Nocardia* spp, and were most frequently isolated in mid lactation. *Mannheimia* spp. was isolated at a low prevalence, 5% or less, throughout lactation, and isolates were from the same two udder halves of two ewes. There was a peak in the isolation of coliforms in the second week of lactation (26% of samples collected) although this was also the week of peak prevalence of contaminated samples. The prevalence of CNS was almost constant throughout the first 6 weeks of lactation with at least 70% of milk samples yielding growth, but then decreased in weeks 7 and 8 with 44% and 53% of samples yielding CNS. *Bacillus* spp. showed a similar pattern to CNS in that it decreased in prevalence in weeks 7 and 8, but, although its prevalence was generally high over the preceding weeks, it was more variable in prevalence than CNS. Observations of *Proteus* spp. and *Streptococcus* spp. were too infrequent to describe a pattern. The frequency of contaminated samples was highest at the beginning of lactation and rare at the end of lactation. The percentage of samples from which no bacteria was cultured was highest at the end of lactation, in 12% and 19% of samples collected in weeks 7 and 8 of lactation respectively, compared to only 4-5% of samples collected in the first 3 weeks of lactation (Figure A.2.4.).

Figure 2.5 Frequency of isolation of four bacteria species by week of lactation



2.4.7 Bacterial species isolated across ewe age groups

There was no particular pattern of isolation of particular bacteria species across most ewe age groups (Table A.2.4; Figure A.2.5). There were too few observations of *Streptococcus* spp. or *Proteus* spp. to comment on a pattern although for both of these bacteria they were present in only two age groups; most *Streptococcus* isolates were from subsequent observations of the same ewe (ewe 117) (Figure 2.8 and Figure 2.9) whereas *Proteus* was observed in different ewes. The majority (31/36 (86.1%)) of observations of *Mannheimia* were from an udder half of two different 8 year old ewes (ewes 33 and 36). *Nocardia* was present in most age groups but slightly more prevalent in 8 year old ewes. Coliforms appeared to be slightly more common in one year old and 8 year old ewes. There was a slight trend for increased prevalence of *S. aureus* in older ewes (5 years old and older) and isolates were often from the same ewe in one or both halves across successive weeks (ewes 1, 13, 37,47, 49). *Bacillus* and CNS were similarly highly prevalent (approximately 60-70%) in all age groups, whereas *Corynebacteria*, Gram negative bacteria and *Nocardia* spp. did not exhibit a particular pattern with respect to ewe age and were at lower prevalence (approximately 6-20%) across all age groups. The proportion of milk samples yielding no growth was fairly constant across all age groups, with a slight overrepresentation of middle age ewes (3-5 yrs old). The prevalence of milk samples that were contaminated did not show any particular pattern with ewe age.

2.4.8 Level of growth of bacteria species

Most observations of growth of bacteria from milk samples were classified as scant. Moderate growths were observed more frequently from milk samples collected in weeks 0 and 1 of lactation whilst heavy growths were observed from milk samples collected in the weeks 2 and 3 of lactation. Observations of scant growth accounted

for a greater proportion of bacterial growths in the second half of lactation than earlier on in lactation (Table 2.10, Figure 2.6). Across ewe age groups, the proportion of growths classified as scant was similar. Heavy growths were observed more frequently from milk samples from one, five and eight year old ewes (Table 2.11, Figure 2.7). The lowest proportion of milk samples yielding heavy growth were collected in week 5 of lactation and from 3 year old ewes. In general, moderate or heavy growths were observed from milk samples collected earlier on in lactation and from older ewes.

Although observations of *Mannheimia* spp. and *Proteus* spp. were uncommon, growth, when observed, was moderate or heavy (Table 2.12). Observations of *Bacillus* spp. and CNS were very common and growth was mostly scant. *Nocardia* spp., *Corynebacterium* spp. and *Staphylococcus aureus* were observed occasionally and mostly growth was scant. Coliforms were infrequently isolated and the majority of observations of growth were classified as scant but there was less of a difference between levels of growth of coliforms than for other bacteria.

Table 2.10 Number (%) of observations of heavy, moderate or scant growth of bacteria species by weeks in lactation

Weeks in lactation	Scant growth n	%	Moderate growth n	%	Heavy growth n	%	% of heavy or moderate growth	Total obs of bacteria
0	116	74.4	25	16.0	15	9.6	25.6	156
1	139	69.9	47	23.6	13	6.5	30.1	199
2	141	81.6	9	5.2	23	13.3	18.5	173
3	121	77.6	9	5.8	26	16.7	22.5	156
4	154	89.0	5	2.9	14	8.1	11.0	173
5	146	88.0	14	8.4	6	3.6	12.0	166
6	108	88.5	7	5.7	7	5.7	11.5	122
7	49	84.5	3	5.2	6	10.3	15.5	58
8	58	90.6	0	0.0	6	9.4	9.4	64
All Weeks	1032	81.5	119	9.4	116	9.2	18.6	1267

Figure 2.6 Percentage of observations of scant, moderate or heavy growth of bacteria species by weeks in lactation

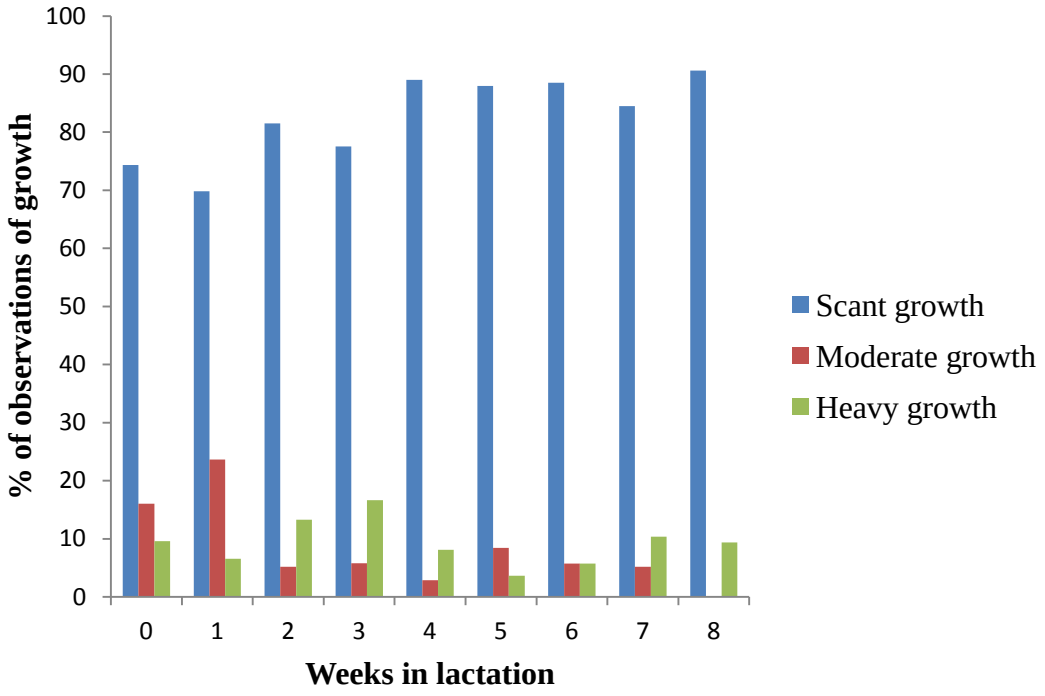


Table 2.11 Numbers (%) of observations of heavy, moderate or scant growth of bacteria species by age of ewe

Age of ewe (yrs)	Scant growth n	%	Moderate growth n	%	Heavy growth n	%	% of heavy or moderate growth	Total no. of obs of bacteria
1	118	78.7	4	2.7	28	18.7	21.4	150
2	402	83.6	39	8.1	40	8.3	16.4	481
3	241	80.3	45	15.0	14	4.7	19.7	300
4	156	82.5	18	9.5	15	7.9	17.4	189
5	42	77.8	4	7.4	8	14.8	22.2	54
8	22	71.0	2	6.5	7	22.6	29.1	31
10	51	82.3	7	11.3	4	6.5	17.8	62
All ages	1032	81.5	119	9.4	116	9.2	18.6	1267

Figure 2.7 Percentage of observations of heavy, moderate or scant growth of bacteria species by age of ewe

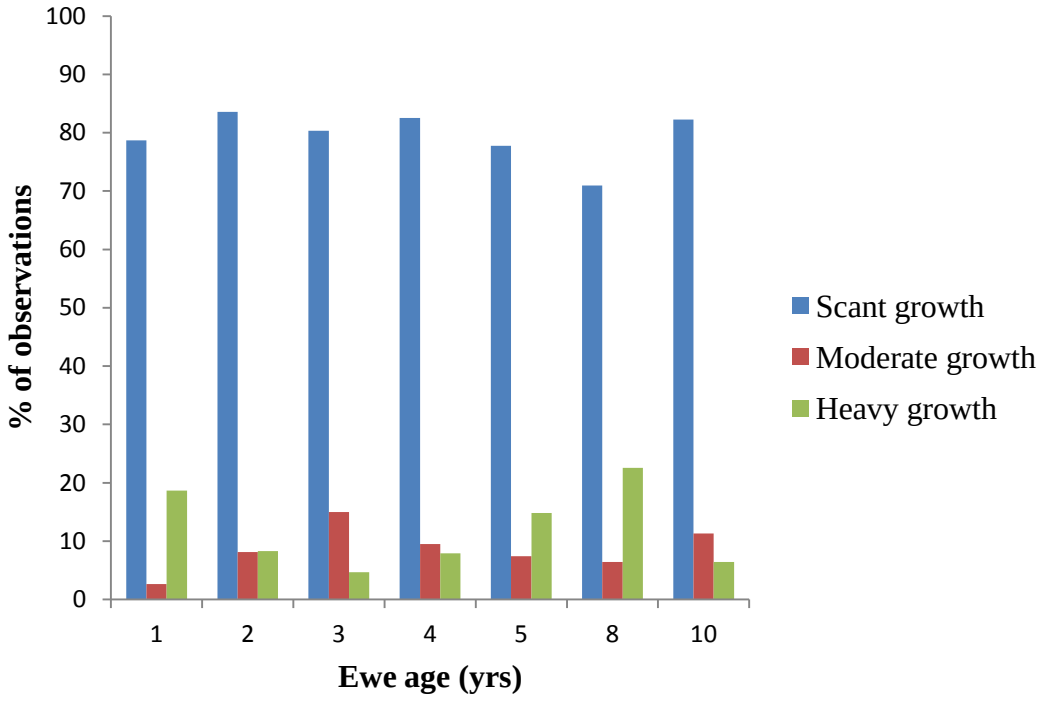


Table 2.12 Numbers (%) of scant, moderate or heavy growth observed for each bacteria species

Bacteria	Observations of bacteria growth						
	scant		moderate		heavy		Total
	n	%	n	%	n	%	N
Bacillus	373	85.35	42	9.61	22	5.03	437
Coliforms	27	64.29	8	19.05	7	16.67	42
Corynebacterium	69	82.14	11	13.10	4	4.76	84
Gram negative	77	77.78	8	8.08	14	14.14	99
Mannheimia	0	0.00	2	20.00	8	80.00	10
Nocardia	51	94.44	2	3.70	1	1.85	54
Proteus	0	0.00	1	20.00	4	80.00	5
CNS	397	81.35	42	8.61	49	10.04	488
Staph aureus	36	80.00	3	6.67	6	13.33	45
Streptococcus	2	66.67	0	0.00	1	33.33	3

2.4.9 Longitudinal patterns of bacteria and somatic cell count.

Bacillus and CNS were commonly isolated together and were frequently isolated from most udder halves, although there were a few exceptions (left udder halves of ewes 47, 12 and 33, Figure 2.8).

Mannheimia spp. was isolated from only two udder halves, in different ewes. In one udder half, isolations were over successive weeks (left half of ewe 33), whereas in the other udder half it was absent from culture for three consecutive visits in mid lactation before being re-isolated the end of lactation (left half of ewe 36). Gram negative bacteria were sometimes intermittently isolated and sometimes persistently isolated from the same udder half (ewes 34 and 59, Figure 2.9), but there was a greater tendency for it to be isolated more than once from the same half (ewes 13 and 45).

There was high variability of SCC in some udder halves. Higher somatic cell counts were seen in udder halves at the same observation of *Mannheimia* spp., *Proteus* spp. and, in some but not all instances, *S. aureus*. Where these bacteria species had been isolated but were not present the subsequent week, there was a fall in HSCC the subsequent week.

Figure 2.8 Trend of log_{10} HSCC over weeks with presence of bacteria in the left udder half of each ewe

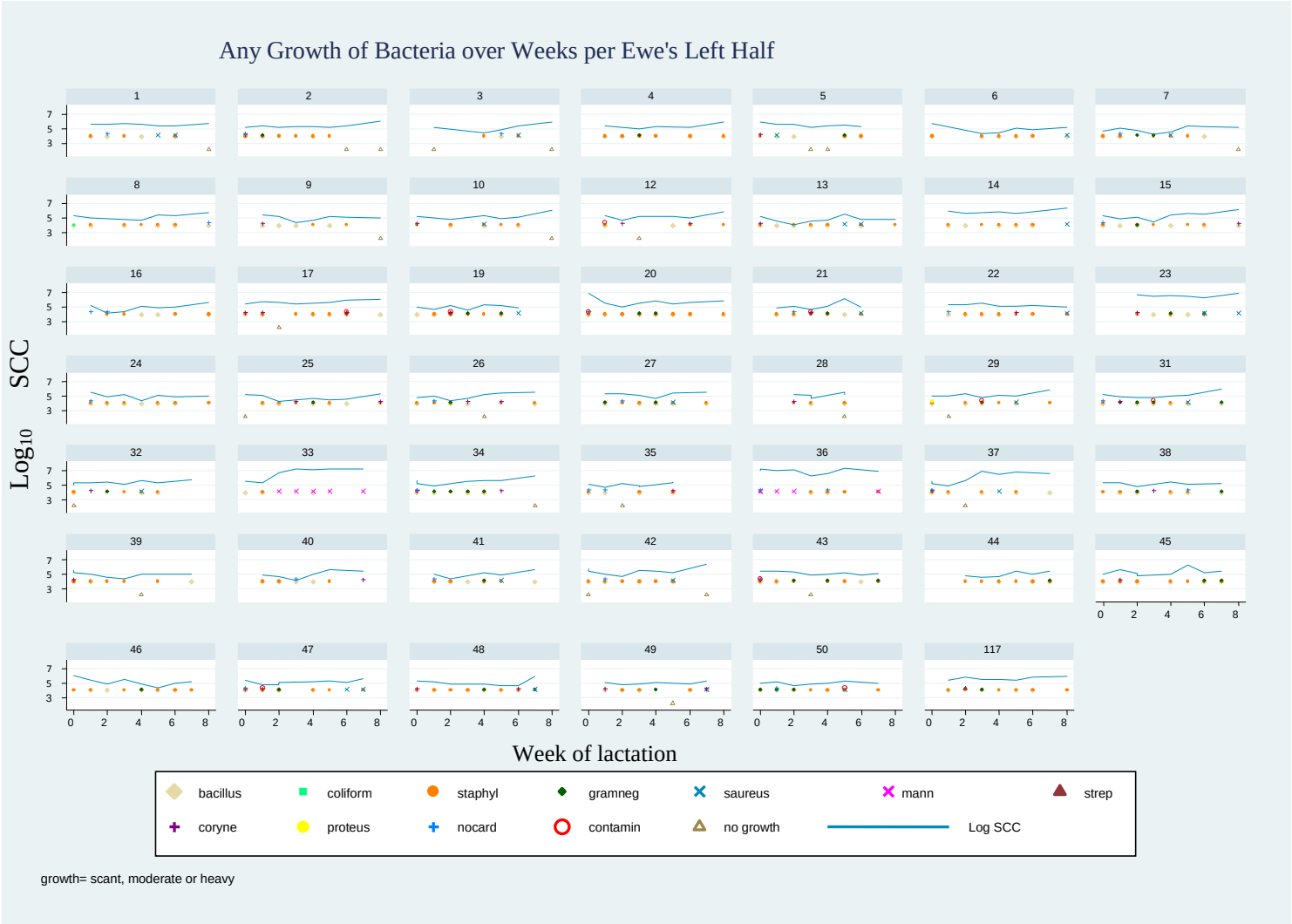
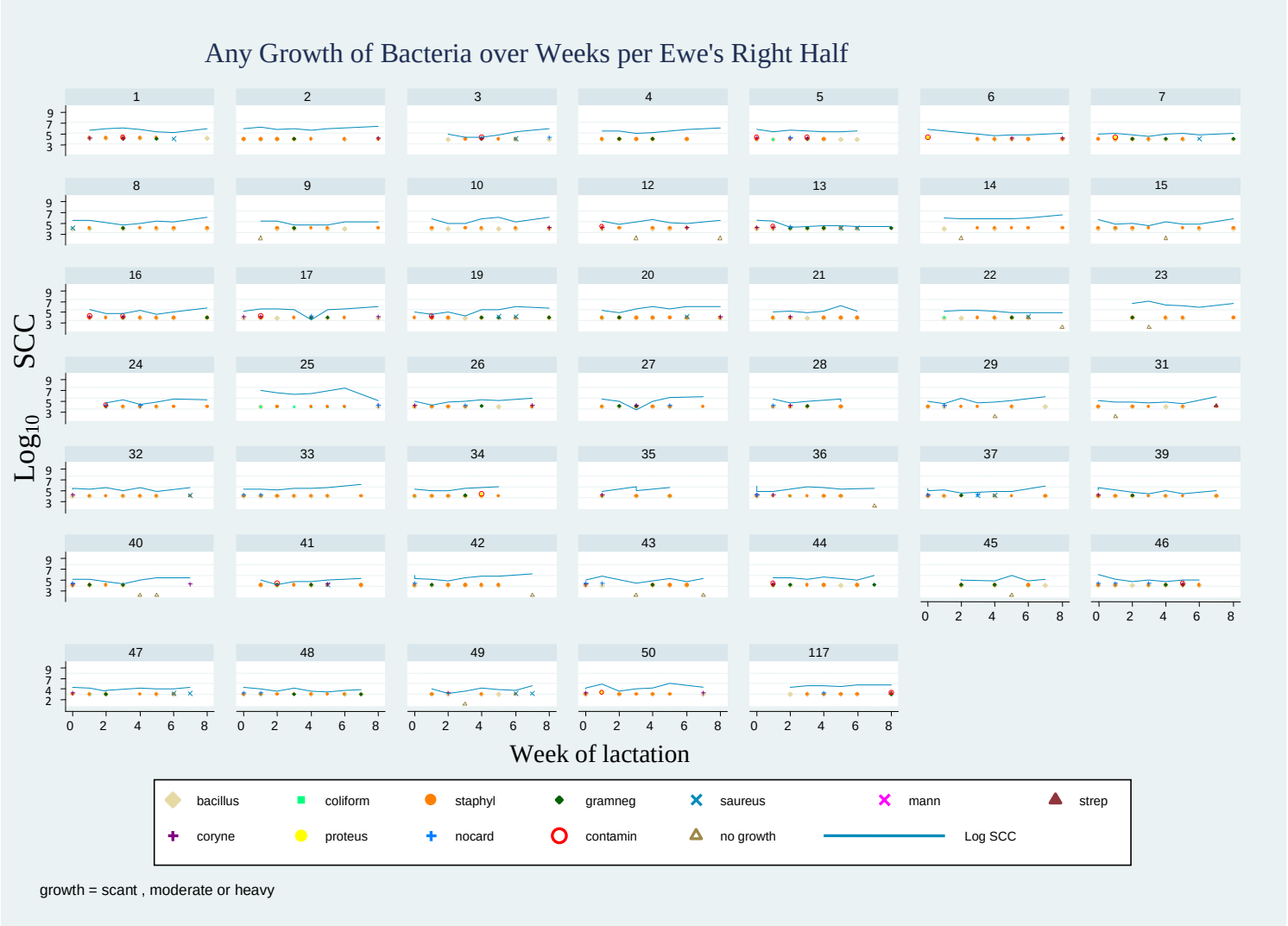


Figure 2.9 Trend of log_{10} HSCC over weeks with presence of bacteria in the right udder half of each ewe



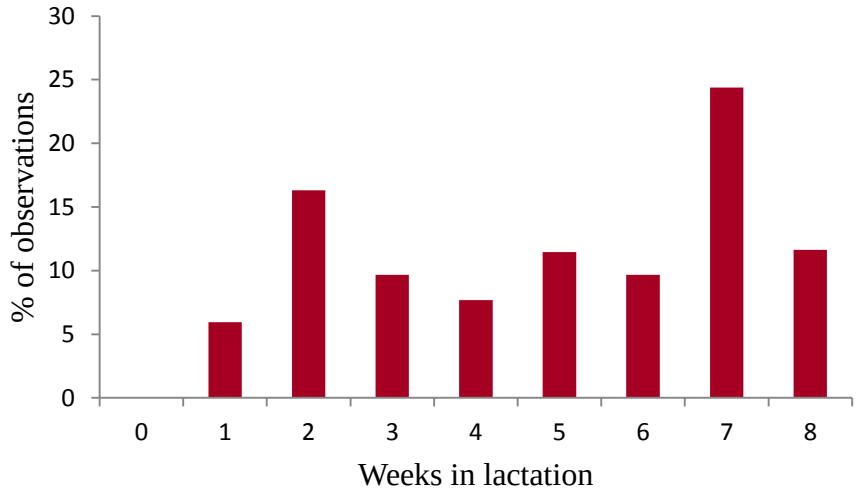
2.4.10 Teat lesions

There was a peak of observations of new teat lesions in week 7 of lactation when a new teat lesion was observed on almost a quarter of udder-halves examined. After the first visit, when no teat lesions were observed, the frequency of observations of teat lesions was fairly similar across weeks (8-15%) until the eighth visit when a higher proportion of teat lesions was also observed. Most ewes were in week 7 of lactation at the eighth visit. There was another, lower peak of observations in week 2 of lactation (Table 2.13, Figure 2.10).

Table 2.13 Incidence of teat lesions by weeks in lactation

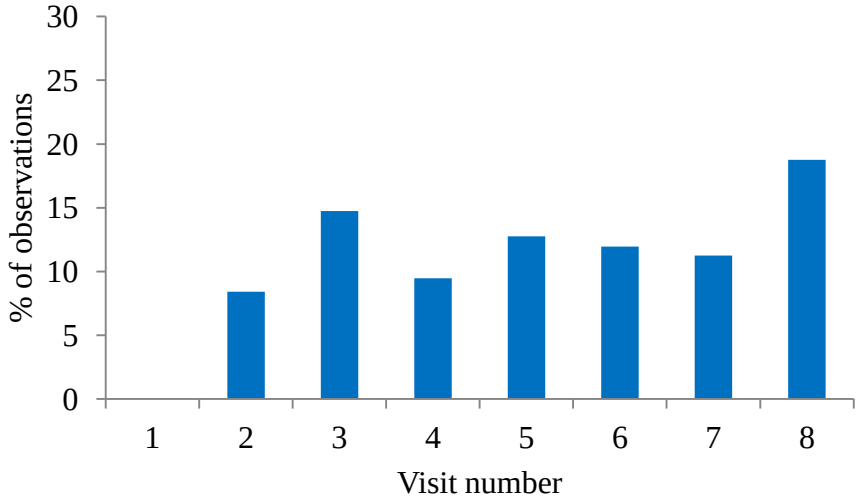
The observation of a new teat lesion over weeks and visits							
Wk	No. halves with new teat lesion	No. halves observed	% of obs	Visit	No. halves with new teat lesion	No. halves observed	% of obs
0	0	67	0.00	1	0	95	0.00
1	5	84	5.95	2	8	95	8.42
2	15	92	16.30	3	14	95	14.74
3	9	93	9.68	4	9	95	9.47
4	7	91	7.69	5	12	94	12.77
5	11	96	11.46	6	11	92	11.96
6	6	62	9.68	7	8	71	11.27
7	10	41	24.39	8	6	32	18.75
8	5	43	11.63				
All weeks	68	669	10.16	All visits	68	669	10.16

Figure 2.10 Percentage of observations each week in lactation where a new teat lesion was recorded



The distribution of teat lesions by visit was more uniform (Figure 2.11), thus the variation of teat lesion prevalence was explained by days in lactation rather than by visit.

Figure 2.11 Percentage of observations each visit where a new teat lesion was recorded



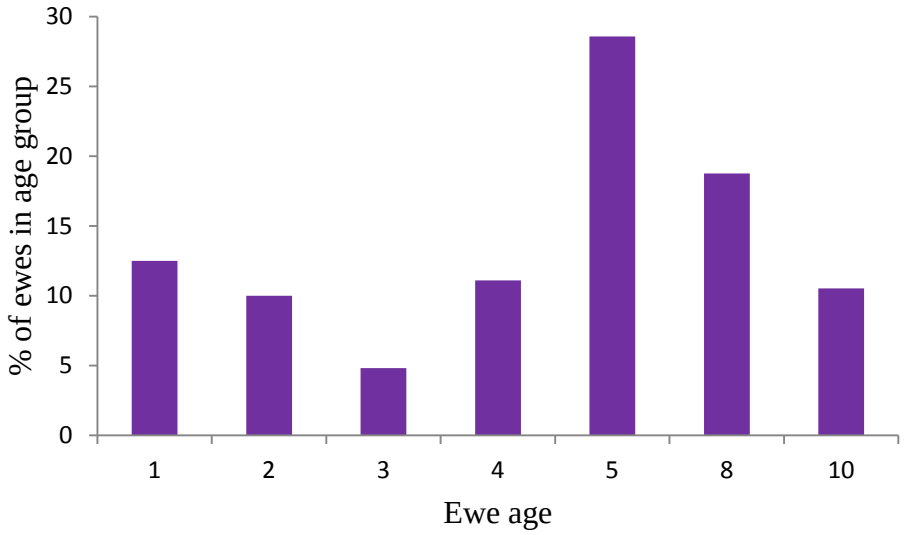
The incidence of new teat lesions over the nine week observation period was 10.2% (68 new teat lesions from 669 observations). There was a significant difference in the incidence of teat lesions by week of lactation (Pearson χ^2 (8) = 23.02, p = 0.003)

and visit (Pearson χ^2 (7) = 16.99, p = 0.017). The incidence of new teat lesions was significantly different between ewes of different ages (Pearson χ^2 (6) = 17.41, p = 0.008). The incidence of new teat lesions was highest in 5 year olds (29%, n = 8) and eight year olds (19%, n = 3) although fewer udder halves of ewes of 5 years of age or older were observed for teat lesions (Table 2.14). Three year old ewes had the lowest incidence (5%) of teat lesions.

Table 2.14 Incidence of teat lesions by age of ewe

Ewe age	No. observations of new teat lesions	No. udder half observations	% of obs
1	9	72	12.50
2	25	250	10.00
3	8	166	4.82
4	11	99	11.11
5	8	28	28.57
8	3	16	18.75
10	4	38	10.53
Total	68	669	10.16

Figure 2.12 Distribution of new teat lesions by ewe age



2.4.11 Correlation of variables

The observation of a new teat lesion on an udder half and the observation of any, including prexisting, teat lesion on the udder half at that observation were strongly correlated ($r = 0.57$, $p < 0.05$). The observation of new teat lesion on an udder half and the occurrence of a teat lesion over the entire observation period on an udder half were moderately correlated with each other ($r = 0.34$, $p < 0.05$) (Table 2.15). Observation of *Staphylococcus aureus* showed slight to moderate correlation with time in lactation and the teat lesion variables.

Table 2.15 Pairwise correlations of teat lesion observations with ewe level variables and with bacteria presence

	Ewe age	Litter size	Weeks	Days	Teat lesion on udder half (Prev)	Teat lesion on udder half (Inc)	Teat lesion on udder half ever
Ewe age	1						
Litter size	-0.09*	1					
Weeks	0.07	-0.04	1				
Days	0.058	0.03	0.99*	1			
Teat lesion on udder half (Prevalence)	0.15*	0.07	0.34*	0.38*	1		
Teat lesion on udder half (Incidence)	0.04	0.08*	0.10*	0.13*	0.57*	1	
Teat lesion on udder half ever	0.11*	0.14*	-0.03	0.01	0.59*	0.34*	1
Bacillus	-0.07	0.03	-0.16*	-0.17*	-0.11*	-0.10*	0.00
Coliform	-0.06	-0.06	-0.17*	-0.19*	-0.08*	-0.05	-0.06
Corynes	-0.01	0.05	-0.15*	-0.16*	-0.09*	-0.01	-0.01
Fungal	-0.04	-0.02	-0.01	-0.01	-0.06	-0.07	-0.05
Gve	-0.08*	-0.02	0.00	0.01	-0.03	0.01	0.00
Nocardia	-0.01	0.09*	-0.23*	-0.23*	-0.07	-0.03	-0.00
Mannheimia	0.17*	0.12*	-0.03	-0.00	0.15*	0.04	0.12*
Proteus	0.00	-0.05	-0.09*	-0.08*	-0.01	-0.03	0.05
Cns	-0.05	0.028	-0.10*	-0.10*	-0.01	-0.05	0.08*
Saureus	0.04	-0.08	0.23*	0.23*	0.25*	0.23*	0.13*
Strep	0.017	-0.01	0.06	0.06	0.01	-0.02	-0.02

* indicates significance $p < 0.05$

All bacterial species and variables were retained for building the model except for weeks in lactation which was almost perfectly correlated with days in lactation.

2.4.12 Univariable analysis

In the univariable analysis, using a three-level hierarchical model structure to account for clustering within visit and repeated measures of udder half and ewe, $\log_{10}$ HSCC was significantly associated with days in lactation and the quadratic of days in lactation. Variables significantly associated with a lower $\log_{10}$ HSCC were no growth of bacteria at that visit, the observation of CNS, *Bacillus* spp., Gram negative bacteria at that visit, or the observation of *Nocardia* spp. at the previous visit. Significantly higher $\log_{10}$ HSCC was observed in ewes of six years and older and in udder halves that had a teat lesion at any point over the observation period. The observation of *Staphylococcus aureus* at a previous visit and the observation of a teat lesion at a previous visit (Table 2.16) were also significantly associated with a higher $\log_{10}$ HSCC in the univariable analysis. The observation of *Mannheimia spp.* was only made from two ewes and although was significantly associated with a higher in $\log_{10}$ SCC in the udder half, robust conclusions could not be drawn from so few observations. Similarly, observations of *Streptococcus* spp. and *Proteus* spp. were made from too low a number of ewes for robust comparisons although there was no significant association of these bacteria with HSCC in the univariable analysis.

Table 2.16 Univariable analysis in 3-level model of log_{10} HSCC

Variable	Coefficient	95% CI	
		lower	upper
Response Variable is log_{10}SCC			
Intercept in null 3-level model	5.31	5.21	5.41
Days in lactation*	5.7×10^{-3}	3.8×10^{-3}	7.6×10^{-3}
Days in lactation^2*	1.4×10^{-4}	1.1×10^{-4}	1.7×10^{-4}
Weeks in lactation*			
0	Reference		
1	-0.13	-0.25	-0.01
2	0.32	0.20	0.44
3	-0.33	0.45	-0.22
4	-0.20	-0.32	-0.09
5	-0.06	-0.18	0.05
6	-0.16	-0.29	-0.02
7	-0.04	-0.11	0.18
8	0.23	0.08	0.38
Age of ewe (yrs)*			
2 years or less	Reference		
3 to 5 years	0.09	-0.09	0.27
6 years or more	0.71	0.39	1.03
Number of lambs rearing			
1	Reference		
2	-0.27	-0.68	0.14
3	-0.39	-0.95	0.18
Observation of at least one teat lesion on udder half through observation period (period prevalence of udder halves with teat lesions)*			
No	Reference		
Yes	0.12	0.03	0.22
Observation of a new teat lesion at that visit (incident of teat lesion)			
No	Reference		
Yes	-0.05	-0.16	0.06
Observation of a teat lesion at the previous visit*			
No	Reference		
Yes	0.154	0.04	0.27
Observation of a new teat lesion at the previous visit (incident of teat lesion)			
No	Reference		
Yes	-0.05	-0.18	0.08
Presence of bacteria at that observation			
Coagulase negative Staphylococcus spp.*			
No	Reference		
Yes	-0.152	-0.23	-0.08
Bacillus spp.*			

No	Reference		
Yes	-0.093	-0.16	-0.02
Gram negative bacteria*			
No	Reference		
Yes	-0.185	-0.28	-0.09
Corynebacteria spp.			
No	Reference		
Yes	-0.01	-0.11	0.09
Nocardia spp.			
No	Reference		
Yes	-8.5×10^{-3}	-0.13	0.11
Staphylococcus aureus			
No	Reference		
Yes	0.124	-0.01	0.26
Coliform bacteria			
No	Reference		
Yes	-0.058	-0.08	0.20
Pasteurella/Mannheimia spp.*			
No	Reference		
Yes	1.274	0.01	1.64
Proteus spp.			
No	Reference		
Yes	0.36	-0.02	0.74
Streptococcus spp.			
No	Reference		
Yes	0.485	-0.01	0.98
Contaminated			
No	Reference		
Yes	0.083	-0.08	0.25
No growth*			
No	-0.166	-0.30	-0.03
Yes	Reference		
Presence of bacteria at previous observation			
Coagulase negative Staphylococcus spp. lag			
No	Reference		
Yes	-0.01	0.10	-0.08
Bacillus spp.lag			
No	Reference		
Yes	-0.06	-0.14	0.01
Gram negative bacteria lag			
No	Reference		
Yes	-0.10	-0.20	0.00
Corynebacteria spp.lag			
No	Reference		

Yes	0.02	-0.09	0.13
Nocardia spp. lag*			
No	Reference		
Yes	-0.14	-0.27	-0.01
Staphylococcus aureus lag*			
No	Reference		
Yes	0.38	0.23	0.54
Coliform bacteria lag			
No	Reference		
Yes	-0.10	-0.25	0.05
Pasteurella/Mannheimia spp. lag*			
No	Reference		
Yes	1.03	0.63	1.43
Proteus spp. lag			
No	Reference		
Yes	-0.15	-5.42	2.50
Streptococcus spp. lag			
No	Reference		
Yes	-0.18	-1.06	0.71
Contaminated lag			
No	Reference		
Yes	-0.09	-0.26	0.08
No growth lag			
No	Reference		
Yes	-0.07	-0.23	0.10

	Variance	**95% CI**	
		lower	**upper**
Between ewe residual variance	0.076	0.021	0.131
Between udder-half residual variance	0.076	0.348	0.117
Between visit residual variance	0.170	0.150	0.190

*** denotes was significant in univariable analysis**

2.4.13 Multivariable analyses

Two three-level multivariable models were constructed with $\log_{10}$ HSCC as the continuous dependent variable. In the first model, the expected mean $\log_{10}$ HSCC at lambing was 3.85 (a HSCC of 723 x 10^3 cells/ml (95% CI: 266 x 10^3, 1,7939 x 10^3)) after controlling for the effects of days in lactation which followed a quadratic and a cubic (Table 2.17). Ewes older than five years of age had significantly higher $\log_{10}$ HSCC of 4.5 (or a HSCC of 3, 420 x 10^3 cells/ml (95% CI: 1, 654 x 10^3, 6, 734 x

10^3)), which was 2, 698 x 10^3 cells/ml higher than the HSCC of ewes that were 5 years of age or less. Two variables that were significantly associated with $\log_{10}$ HSCC in the univariable analysis became insignificant in the multivariable analysis. These were no growth at that visit and the observation of a new teat lesion at the previous visit. Number of lambs reared and observation of a teat lesion had no significant association with HSCC.

Table 2.17 Multivariable model of $\log_{10}$ HSCC by days in lactation and ewe age

Variable	**Multivariable coefficient**	**95% CI**	
		lower	upper
Response variable is Log_{10} HSCC			
Intercept	3.85	3.49	4.22
Days in lactation	-0.05	-0.06	-0.03
Days in lactation^2	1 x 10^{-3}	1 x 10^{-3}	1 x 10^{-3}
Days in lactation^3	-1 x 10^{-5}	-2 x 10^{-5}	-4 x 10^{-6}
Ewe is 5 years old or younger	Reference		
Ewes is older than 5 years	0.65	0.33	0.96
	Variance	95% CI	
		lower	upper
Between ewe residual variance	0.05	0.00	0.09
Between udder half residual variance	0.08	0.04	0.12
Between visit residual variance	0.13	0.11	0.15
-2 x log likelihood: 725.738		(669 out of 669 cases used)	

The second three-level hierarchical model demonstrated that $\log_{10}$ HSCC was also explained by bacteria presence (Table 2.18). The expected mean $\log_{10}$ HSCC when all isolated bacteria types were taken into account was 4.41 (a HSCC of 2, 763 x 10^3 cells/ml (95% CI: 2, 118 x 10^3, 3, 580 x 10^3). Significantly lower somatic cell counts were observed when Coagulase negative *Staphylococcus* spp. (652 x 10^3 cells/ml lower) or Gram negative bacteria (235 x 10^3 cells/ml lower) were isolated from the udder half. Significantly higher somatic cell counts were observed when *Mannheimia* spp. or *Streptococcus* spp. were isolated from the udder half, although these isolations were from a very small number of ewes (two and four ewes respectively)

so robust conclusions could not be drawn for the association of these two bacteria species and SCC. Coefficients and significance of bacterial variables did not change when the variable for ewe age older than five years was added into the model although the expected mean of SCC was lower (log_{10} SCC of 3.79, or SCC of 618 x 10^3 cells/ml) with the inclusion of this age variable.

Table 2.18 Multivariable model of log_{10} HSCC with bacterial infection

Variable	Multivariable coefficient	95% CI	
		lower	upper
Response variable is log_{10} HSCC			
Intercept	4.41	4.29	4.52
Coagulase negative Staphylococcus spp.			
No	Reference		
Yes	-0.12	-0.19	-0.04
Bacillus spp.			
No	Reference		
Yes	-0.04	-0.11	0.03
Gram negative bacteria			
No	Reference		
Yes	-0.16	-0.25	-0.07
Corynebacteria spp.			
No	Reference		
Yes	0.02	-0.07	0.12
Nocardia spp.			
No	Reference		
Yes	-0.01	-0.12	0.11
Staphylococcus aureus			
No	Reference		
Yes	0.09	-0.04	0.22
Coliform spp.			
No	Reference		
Yes	0.11	-0.02	0.25
Pasteurella/Mannheimia spp.			
No	Reference		
Yes	1.19	0.83	1.55
Proteus spp.			
No	Reference		
Yes	0.32	-0.05	0.68
Streptococcus spp.			
No	Reference		
Yes	0.54	0.05	1.02

	Variance	95 % CI	
		lower	upper
Between ewe residual variance	0.07	0.03	0.11
Between udder-half residual variance	0.04	0.02	0.07
Between visit residual variance	0.16	0.14	0.17
-2 x log likelihood: 813.214	(669 out of 669 cases used)		

For both multivariable models residuals and standardised residuals were normally distributed (A.2.6 and A.2.7) and the models provided a good fit to the data. In both models, the residual variances at each level were significant but low. This indicates that, for the model including days in lactation and ewe age, variation of HSCC between visits, between udder halves and between ewes was only partly explained. Likewise, for the model with bacteria variables only, there was still some unexplained variation between visits, between udder halves and between ewes.

2.5 DISCUSSION

2.5.1 Variables associated with somatic cell count

We sought to improve the understanding of udder infection and the dynamics of SCC in suckler ewes by investigating bacterial presence and other factors associated with higher or lower HSCC during the first 10 weeks of lactation. The main findings of the study were that HSCC followed a quadratic and cubic relationship with days in lactation over the first 10 weeks of lactation. Udder half somatic cell count was 2,698 x 10^3 cells/ml higher in ewes that were older than 5 years of age. The variation of HSCC could also be explained with the presence of culturable bacteria. Coagulase negative *Staphylococcus* spp. and Gram negative infections were associated with lower HSCC but did not explain much of the variation in SCC between udder halves. Udder half somatic cell count was not significantly associated with teat lesions. We observed a temporal pattern for the isolation of some species of bacteria over lactation; *Corynebacteria* spp. and *Nocardia* spp. decreased in prevalence over mid lactation whilst most observations of coliforms were in week 1 of lactation. Almost all observations of *S. aureus* were in the second half of lactation. CNS and *Bacillus* were ubiquitous across all weeks. The temporal pattern of bacteria isolation necessitated two models to be built to explore factors associated with somatic cell count, lest the effect of bacteria be lost by the inclusion of days in lactation.

This is the first study to quantitatively assess the longitudinal pattern of milk SCC of udder halves of suckler ewes through lactation whilst investigating and accounting for the association of HSCC with bacterial infection, ewe age and the presence of teat lesions using a hierarchical model structure to account for clustering of the data due to repeated measures within udder half of ewes. Hariharan *et al.* (2004) performed a similar longitudinal study investigating HSCC and bacterial infection of the udder of

50 suckler ewes over 10 weeks of lactation in a Scottish flock and although the effect of ewe age was accounted for, teat lesions were not recorded. To assess the association of pathogen the authors grouped the bacteria into minor or major pathogens and the relationship with ln SCC did not appear to account for repeated measures of the data within ewes. Watkins *et al.* (1991) performed a longitudinal study of ewes in seven flocks in Southern England, recording age of ewe and the presence of teat lesions but did not directly count somatic cells, instead used the whiteside test (WT) as an indication of level of SCC, the relationships being assessed with prevalence of a predetermined definition of subclinical mastitis (WT positive and bacteriology positive milk sample).

2.5.2 Temporal patterns of somatic cell count

This was not the first study to demonstrate that SCC exhibits a temporal pattern in suckler ewes. Hariharan *et al.* (2004) demonstrated significantly higher ln SCC in the first 2 weeks of lactation than in later weeks up to the tenth week of lactation. In dairy ewes, Fuertes *et al.* (1998) recorded a lower SCC in week 5 of lactation, which coincided with maximum milk yield. A similar pattern has been observed in dairy cows over a longer lactational period, with highest SCC at the start of lactation and a decrease in SCC at 50 days in lactation and a gradual rise again towards the end of lactation (de Haas *et al.*, 2002). Temporal patterns of SCC can by explained by physiological variables such as milk yield but also influenced by infection status. The change in SCC over lactation may be described in part by the dilution effect of increased yield with lactation stage from parturition (Green *et al.*, 2006). Although the milk yield of suckler ewes compared to dairy sheep and dairy cows is substantially lower, quantification of the milk yield of study ewes was out-with the scope of this study. Instead we investigated the effect of increased yield by

accounting for litter size as a proxy variable for milk yield, since ewes rearing twins are likely to experience increased milk demand, and hence produce a larger milk yield over lactation. However, rearing more than one lamb was not significantly associated with lower HSCC in either the univariable analysis or multivariable analysis and was therefore not included in the final model.

2.5.3 Age of ewe

Ewes that were older than 5 years of age had a HSCC that was 2, 250 x 10^3 cells/ml higher than ewes that were 5 years old or younger. However, although significant, this was based on relatively few observations of older ewes; there were only four ewes above the age of 5 years (one 8 year old and two 10 year olds). When strata were merged to include two ewes of 5 years of age, the effect of age was still significant although a less good model fit was provided. Our findings are in agreement with Hariharan *et al.* (2004) who also observed a higher SCC in older suckler ewes. In Hariharan's study, the oldest ewes in the study group, which were seven years of age had significantly higher ln SCC than younger ewes. Watkins *et al.* (1991) observed higher prevalence of subclinical mastitis in multiparous suckler ewes older than 2 years of age. Higher levels of inflammation in the udder half of older ewes may indicate higher infection levels and previous exposures to mastitis pathogens due to a greater number of lactations. Another explanation is that very old ewes have lower productivity and therefore a lower milk yield and less dilution of somatic cells per millilitre of milk although it was not possible to determine this effect in this study. In dairy ewes, the association of age and SCC has been fairly well documented. Lafi *et al.* (2006) demonstrated higher SCCs in dairy ewes of greater than first parity. A significantly higher frequency of subclinical mastitis, as defined by a positive CMT score and growth of bacteria from milk samples, was

observed in dairy ewes of at least third parity (Beheshti *et al.*, 2010). Gonzalo *et al.* (2002) found parity to be significant when the effect of bacterial variables were not included in the mixed regression model of dairy ewe and bacterial infection variables on SCC. In dairy cows, an increased risk of cow SCC > 199, 000 cells/ml was observed with increased parity; cows of first and second parities had a decreased risk compared to cows of third parity (Breen *et al.*, 2009). In contrast, Paape *et al.* (2007) found that SCC of primiparous dairy ewes was significantly higher than that of ewes of high parity. In our study we did not detect an association between no previous lactation with SCC but there may have been insufficient power to detect any difference due to the low number of primiparous ewes. Although young ewes predominated in the study, ewes were mated at one year old on this farm and all but five ewes had previously had at least one lactation.

2.5.4 Bacteria

The isolation of viable Gram negative bacteria was associated with a HSCC of 431 x 10^3 cells/ml, which was 164 x 10^3 cells/ml lower than that of udder halves without Gram negatives at that observation. This may suggest that udder halves with a SCC of 431 x 10^3 cells/ml cells/ml or lower were at a greater risk of infection with Gram negative bacteria.

Gram negative bacteria associated with udder infection include *Mannheimia haemolytica*, *E. coli*, *Proteus* spp., *Serrati* spp., *Klebsiella* spp. and *Pseudomonas* spp. (Las Heras *et al.*, 1999). In our study, using the one plate culture technique, only *M. haemolytica* and *Proteus* were identified at species level and separately grouped from the more generic classification of coliforms or Gram negative. As there was an inverse pattern of observation of coliforms and Gram negative and because identification of a colony as Gram negative is less specific than identification of a

coliform, some misclassification bias may have occurred, and the true prevalence of coliforms may have been underestimated. However, no association of presence of coliform with HSCC was observed even when observations of coliforms were merged with the more generic category of Gram negatives. Green *et al.* (2004) demonstrated a negative association between mean log SCC and clinical mastitis in cows associated with *E. coli* (and also no growths), thus cows that developed clinical mastitis associated with *E. coli* or in the absence of bacterial growth had lower SCC than cows which did not get clinical mastitis. A finding by Peeler *et al.* (2003) was that dairy cow quarters with low quarter SCC (21-100 x 10^3) were at an increased risk of developing clinical mastitis although the extent of this effect may have been pathogen specific; quarters with low SCC, (6-200 x 10^3 cells/ml) had a decreased odds of developing coliform associated clinical mastitis when compared to those with very low quarter SCC (1-5 x 10^3 cells/ml). This supports the theory of a protective threshold of SCC below which the risk of disease resulting from infection with some bacteria such *E. coli* is increased. In our study the observation of lower HSCC when Gram negatives were present may be explained by the causative bacteria being more likely to cause infection after entry into the udder half when SCC was low, or being initially able to evade, or slow to trigger, an immune response. In our study coliforms were mostly isolated in week 1 of lactation which could indicate infection in early lactation but could also be indicative of infection acquired during, or persisting throughout, the dry period; in dairy cows, mastitis in early lactation can represent a chronic dry period coliform infection (Bradley and Green, 2000). Coliforms, when present in our study (6.3% (n=42) of observations of bacteria), represented relatively high proportions of moderate or heavy growth compared to other bacteria, although it is not known whether this was due to the

manner of growth culture plate, rather than a true reflection of intramammary bacteria load.

The observation of CNS was significantly associated with lower HSCC. In contrast, Leitner *et al.* (2003) demonstrated higher SCC when CNS were isolated from dairy ewe milk samples and Gonzalo *et al.* (2002) demonstrated higher SCC associated with novobiocin-sensitive CNS bacteria but not with novobiocin-resistant CNS bacteria. In our study, no attempt was made to further speciate the CNS identified. There is a theory that CNS bacteria provoke a slight immune response that help prevent disease by keeping an active polymorph community in the udder which are primed to fight other invading, more severe pathogens. This theory has some evidence to support it; in an experimental study of the effect of CNS in the teat duct of ewes on the outcome of challenge with *M. haemolytica* infection, udder halves with a high growth of CNS had fewer observations of bacterial flora post challenge than udder halves that weren't challenged or than udder halves that were challenged but had prior bacterial growth. This suggested that heavy colonisation of udder halves with CNS may have conferred a protective role for the teat against disease from other competing major pathogens (Fragkou *et al.*, 2007). The high frequency of isolation of CNS in our study and the association of lower SCC with CNS presence suggests that the presence of CNS, when adjusting for IMI with other bacterial species, is of low pathogenicity and may confer a protective effect. However, this may be dependent on bacterial load.

There was no association between the second most commonly isolated bacterial species, *Bacillus*, and HSCC. Because most of our milk samples had CNS or *Bacillus* spp. isolated, the association of the absence of bacterial growth from a sample

provided a potential useful variable for comparison. However, whilst no-growth was significantly associated with a lower HSCC in the univariable analysis there was no association when other variables were controlled for. There is some evidence that intermittent shedding with udder halves persistently infected with CNS may occur (Burriel *et al.*, 1997). If this was the case in our study, an absence of bacterial growth may not have been truly reflective of an absence of infection, which may explain why the no-growth variable was not associated with lower HSCC in the multivariable analysis. In our study, we have interpreted the high prevalence of *Bacillus* spp. as being true udder infection. However, it is possible that *Bacillus* spp. was present as contaminant, despite the meticulous sterilisation of the teat and measures taken to collect samples as aseptically as possible. Researchers of udder infection of dairy cows consider low levels (<9 colonies) of *Bacillus* spp. to be a contaminant (A. Bradley, personal communication).

Mannheimia spp. and *Streptococcus* spp. were both significantly associated with a small increase in HSCC although there were too small a number of observations of these bacteria to robustly assess an association with SCC. Higher WT scores have previously been documented in suckler ewes with *Streptococcus* spp. IMI; some of these ewes subsequently developed clinical mastitis associated with the same *Streptococcus* spp. (Watkins *et al.*, 1991). In the literature, *Mannheimia haemolytica* infection is well documented as being associated with high SCC (Ariznabarreta *et al.*, 2002) and clinical mastitis in ewes (Arsenault *et al.*, 2008; Mork *et al.*, 2007). In our study *Mannheimia* spp. infection was only identified in one udder half of each two ewes. Neither of these two ewes developed clinical mastitis in the study period, although both had high HSCCs at the observations where *Mannheimia* spp. were identified. When isolated, *Mannheimia* yielded heavy growth and was observed on

more than one occasion from the same udder half and thus was considered to represent persistent infection and udder disease. However it was not isolated at every consecutive observation from the infected udder halves in which it was observed which may indicate intermittent secretion of the organism. In a study by Fragkou *et al.* (2008) the organism was consistently cultured from teat duct secretion following experimental inoculation but only intermittently from mammary secretion for the 5 days post lambing. California mastitis test scores were persistently elevated at each observation and damage of the mammary parenchyma noted in necropsy in most ewes. However in a different study the same author demonstrated that whilst experimental inoculation with *M. haemolytica* resulted in clinical mastitis in some ewes, others developed only subclinical disease and there was a breed difference in susceptibility to clinical disease (Fragkou *et al.*, 2007). Experimental infection with *Mannheimia* spp. may thus be considered to be associated with marked and sometimes persistent udder disease although this may not be observed as a clinical case.

Although we did not find any other robust significant associations of bacterial growth with HSCC for bacterial species other than those generically identified as Gram negative bacteria and CNS, the inclusion of all observations of bacteria spp. in the multivariable model explained variation of HSCC. Hariharan *et al.* (2004) found no association between bacteria isolated and SCC although for that study the effect of pathogen was assessed according to classification of bacteria species into two groups, of either minor or major pathogens. Conversely, a study of seven dairy flocks by Gonzalo *et al.* (2002) found that bacterial species accounted for much of the variation on SCC. In contrast to other studies, we cultured bacteria from a high proportion (93.7%) of milk samples collected, with many samples yielding growth of

two (40.5%) or even three (21.7%) species. This suggests that udder infections were common throughout lactation and multiple infections were frequently observed. Most of the bacteria species cultured yielded mainly scant levels of growth. Rather than being reflective of true infection, scant growth of bacteria on culture may reflect inadvertent collection of commensal or environmental bacteria from outside of the teat, which is difficult to avoid in field conditions, despite diligent sterilisation of the teat. If association between SCC and some bacterial species is load dependent, this may explain the absence of an association between HSCC and most of the bacteria species or families observed in the milk. A limitation in our methodology was that the one plate technique used may have lacked sensitivity because some bacteria may not grow well on culture media, or may be competitively inhibited by the presence of others.

In dairy ewes, udder infection with *Staphylococcus aureus* has been associated with an increase in SCC (Gonzalo *et al.*, 2002). However, in our study, presence of *S. aureus* was not significantly associated with higher HSCC in either the multivariable or univariable analysis (although there was borderline significance at 95% level for the univariable analysis). Growth of *S. aureus* only accounted for a low proportion of observations. Eighty percent (n = 36) of cultures of *S. aureus* in our study were of scant growth and only 6 (13%) were of heavy growth. If the effect of *S. aureus* on SCC is load dependent, there may have been insignificant power to detect an association between *S. aureus* and HSCC due to the low number of observations of heavy growth. When looking at individual longitudinal patterns of udder halves of ewes, higher SCC was seen in some udder halves from which *S. aureus* was isolated at the same observation but in udder halves where it was not isolated the subsequent week, there was a fall in SCC the subsequent week, which suggests that some

association of *S. aureus* infection with HSCC may exist. We observed a trend of increasing, then decreasing *S. aureus* infection in the second half of lactation. This may indicate contagious spread, such as via cross-suckling lambs, or alternatively may reflect environmental contamination during milk sampling such as from the skin flora of the teat.

There was no significant association between the isolation of *Proteus* spp. and HSCC although there were insufficient observations of *Proteus* spp. to robustly assess this association. Higher somatic cell counts have previously been associated with *Proteus* infection. For example, in dairy goats, *Proteus* spp. infection was associated with a SCC of 3, 000 x 10^3 cells/ml, compared to 1, 000 x 10^3 cells/ml for CNS and 500 x 10^3 for udder halves yielding no growth (Raynal-Ljutovac *et al.* 2007). In a survey of dairy cows in the states of New York and Pennsylvania, *Proteus* spp. was isolated in 0.3% (n=296) of composite milk samples from cows with IMI (Wilson *et al.* 1997). The prevalence of *Proteus* spp. infection in cow quarters with a SCC>200,00 cell/ml was 0.8%, compared to 0.3% for quarters with clinical mastitis, (Breen *et al.*, 2009). With the exception of one observation in the fifth week of lactation, in our study *Proteus* spp. was only observed in the first two weeks of lactation, which may have been reflective of infection persisting from the dry period rather than new infections acquired in lactation. *Proteus* spp. have been associated with chronic udder infection through the dry period of dairy cows; in a longitudinal study of incidence of bacterial infection through the dry period in six commercial dairy herds, *Proteus* was isolated with greater frequency from quarters in the final three, two, and one weeks of the dry period, at a prevalence of 0.95%, 1.30%, and 1.25% respectively, than at drying off (0.08%) or in the first 100 days of the subsequent lactation (0.52%) (Bradley and Green, 2000).

Although often considered a minor pathogen, *Corynebacteria* spp. has been associated with both subclinical and clinical cases of mastitis (Lafi *et al.*, 1998). In our study, *Corynebacteria* spp. were the fourth most commonly observed bacteria, and although growth was mostly scant (n=69, 82%), *Corynebacteria* spp. accounted for 14.8% (n=99) of all observations of bacterial growth over the observation period and exhibited a clear pattern of decreased frequency in mid lactation. (Bradley *et al.*, 2007) found a similar proportion of quarters (10%) positive for *Corynebacteria* spp. in a survey of dairy cows with clinical mastitis and high SCC on 97 dairy farms. In ruminants without clinical mastitis, isolation rates of *Corynebacteria* have been lower: in less than 0.1 % of dairy cow quarters (Breen *et al.*, 2009) and in 1% of bacteriologically positive, WT positive udder halves of suckler ewes (Watkins *et al.*, 1991). A similar temporal pattern to that observed for *Corynebacteria* spp. was observed with *Nocardia* spp, which were also quite commonly isolated (62 % (n=44) of udder halves) although again growth was mostly scant (94%, n=51). The recovery of *Nocardia* spp. in our study was much higher than in studies of other dairy ruminants. Arsenault *et al.* (2008) recovered *Nocardia* spp. from only 0.4% of bacterially positive udder halves of clinically normal suckler ewes whilst Wilson *et al.* (1997) found the prevalence of dairy cows with *Nocardia* spp. infection to be less than one per cent.

Although we observed a temporal pattern for *Corynebacteria* spp., *Nocardia* spp., Gram negative bacteria and *S. aureus*, no temporal patterns of infection were evident for the other bacterial species cultured nor were any relationships between growth of bacteria species observed. The isolation rate of CNS and *Bacillus* spp. was high across all weeks of lactation. A major limitation of this study was the method of bacterial identification which was a survey approach using a one-step culture method

which may resulted in type 1 and type 2 errors. This method may have lower sensitivity and specificity due to the difficulty or time dependent properties of culture of some species of bacterial organisms or due to operator bias. However this method enabled a high throughput of samples at low cost. A more appropriate method for bacterial identification may have been PCR although the availability of specific PCR and costs for such analysis may have been prohibitive for this project. The findings have led to more work by other researchers at the University of Warwick to further investigate the bacteria presence through PCR development for some of the bacteria species identified in this study and has allowed a more focused and appropriately channelled use of resources.

2.5.5 Teat lesions

We did not find any evidence that teat damage was associated with higher or lower HSCC. This is in concordance with Watkins *et al.* (1991) who did not find any association between presence of teat lesions and subclinical mastitis. However, it was difficult within the scope of our study to adequately distinguish new teat lesions and persistent lesions. By the admission of the author of this thesis, the effect of teat lesion needed a more robust recording approach than was afforded within the practical constraints of the study.

Teat lesions were observed least frequently in 3 year old ewes. Assuming the same level of feeding, this age group may have been less metabolically stressed than younger ewes, which were still growing or rearing their first litter and therefore used more energy for lactation, producing higher yields and greater availability of milk for lambs. Well fed lambs may have been less likely to suckle aggressively resulting in a lower level of teat trauma. Old ewes are generally at a higher risk of chronic disease and feed conversion inefficiency due to advanced dental wear or damage. Ewes in

the oldest age groups in this study may therefore have been less likely to produce sufficiently high yields to meet the nutritional demands of suckling lambs which placed them at a higher risk of teat lesions.

There were two peaks of teat lesions observed. Characterisation of two types of teat lesions, traumatic and non-traumatic has been described (Selin Cooper, Master's thesis, 2011). Cooper described an increase in the incidence of "traumatic" type teat lesions which tended to be acute in onset (bites, tears and pustules) in the third week of lactation and thereafter an increase in frequency of non-traumatic teat lesions which tend to be chronic in nature (scarring) with the peak incidence of non-traumatic teat lesions at 7-10 weeks in lactation. It is plausible that, in the current study, the first peak of teat lesion incidence was of traumatic teat lesions, and the second peak observed in the second study was of non-traumatic teat lesions, although there was no discrimination between teat lesion types at the time of observation. A limitation of our study was that teat lesions were not characterised. This led to further research by Selin Cooper at the University of Warwick resulting in the aforementioned Master's thesis, 2011 (also see Chapter 3).

2.5.6 Litter size

The majority (77%) of ewes reared twin lambs, therefore the effect of litter size was difficult to assess. In addition, a peculiarity of this farm was the production of lambs from highly fecund ewes with large litter sizes but low individual lamb birth weight. Although a litter size of four or five lambs was common, ewes reared a maximum of two or three lambs and any other siblings were removed at 24-48hrs to be reared on an artificial milk replacement. Therefore litter size reared was not the same as number of lambs the ewes gave birth to, which was not recorded, thus the metabolic stress of the ewe in late gestation due to a greater number of lambs *in utero* was not

taken into account. It may be expected that, assuming adequate plane of nutrition, ewes giving birth to and rearing a higher number of lambs would produce a higher milk yield to meet the demand of the suckling lambs thus an effect of dilution of SCC with increased milk yield may result. There was a trend of decreasing HSCC with increased number of lambs reared although this was non-significant. Another limitation of this study was that BCS was not recorded which may have otherwise been used as a variable to account for adequacy of nutritional supply to the ewe.

2.5.7 Independence of udder half somatic cell count

Udder half SCCs were correlated across the interquartile range of SCC in which most observations were made. However, when one udder half had a very high SCC, a correspondingly high SCC was not found in the other half. This suggests that udder halves are independent units and that local inflammatory processes occur in one udder half only. Similarity of udder halves for the majority of the range of SCC was probably due to exposures and factors that were common to both halves, such as ewe level variables (ewe age, litter size, nutritional stress) that resulted in an equal or similar level of somatic cells in each udder half.

2.5.8 Fit of the models

For both multivariable models residuals and standardised residuals were normally distributed and the models provided a good fit to the data. Both models explained the majority of variation between visits, between udder halves and between ewes. However, there was still significant unexplained residual variance at each level in both models. It is possible that, if all of the variables could have been included in the same model, more of the variation between visits, udder-halves and ewes could have been explained. However, the existence of confounding between weeks of lactation and bacterial presence made it necessary to fit two separate models.

2.6 CONCLUSIONS

Udder half SCC followed a temporal pattern with days in lactation and higher SCCs were observed in ewes older than 5 years of age. Presence of bacteria also explained variation of HSCC and lower SCC was associated with the observation of Coagulase negative *Staphyloccus* spp. and Gram negative bacteria in milk collected at the same observation. Somatic cell count between udder halves were well correlated between ranges of 3.7 and 6.3 $\log_{10}$ SCC but at extremes of SCC udder halves became more independent. A key finding was that udder half infection was very common throughout lactation, with temporal patterns of infection for some but not all species of bacteria. Although udder halves were frequently infected with CNS and *Bacillus* spp., level of growth on culture of these pathogens, where present, was low. There were a low number of observations of some major pathogens; although higher HSCCs were observed in two individual ewes with *Mannheimia* spp. infection, these observations were too few to include in the multivariable analysis. A similar observation was made for udder halves with *Streptococcus* spp. infection. We found no association of HSCC with the number of lambs being reared or the observation of a new teat lesion on the udder half at that visit. However, there were limitations due to the high proportion of ewes with twins and accurate characterisation of nature and duration of teat lesions was not attempted. Our understanding of the temporal dynamics of HSCC and suckler ewe udder infection was improved. The study highlighted that further work was necessary to investigate the nature and duration of teat lesions and to further investigate association of ewe age and body condition on HSCC (Chapter 3).

3. CHAPTER 3: A COHORT STUDY OF THE ASSOCIATIONS BETWEEN MILK SOMATIC CELL COUNT, UDDER CONFORMATION AND LAMB WEIGHT IN SUCKLER EWES

3.1 ABSTRACT

In dairy cows and dairy sheep subclinical intramammary infection is associated with a reduction in milk yield. In suckler ewes, intramammary infection has been associated with lower lamb growth rates. However, previous research investigating the relationship between lamb weight and udder infection has not investigated the associations between udder conformation, somatic cell count and lamb weight. A cohort study of 67 ewes from one farm was carried out from January to May 2010. The number and sex of lambs were recorded at lambing. Within two days of lambing and at 14 day intervals for a further four to five occasions a milk sample was collected from each udder half of each ewe for udder half somatic cell count (HSCC), and ewe body condition, teat condition, lamb health and weight were recorded. Ewe udder conformation and teat placement were scored two weeks after lambing.

A multilevel model was constructed with weight of lamb as the outcome variable and three random effects for ewe, lamb and repeated measures of weight. The key results were that lower lamb weight was significantly ($p<0.05$) associated with ewe mean SCC>400,000 cells/ml (-1.70kg), a traumatic teat lesion (a bite, a tear or chapping) present 14 days previously (-0.65kg), being reared in a multiple litter (-1.67kg), presence of diarrhoea at the examination (-1.15kg) and being reared by a ewe that was in BCS of 2.5 or less before lambing compared with a ewe in BCS of 3

or more before lambing(-1.30kg). Higher lamb weight was significantly associated with increasing lamb age in days (0.22kg/day) and higher birth weight (1.56 kg).

A second multilevel model was constructed with $\log_{10}$ HSCC as the outcome variable and with ewe, udder half and repeated measures of SCC as random effects. Higher HSCC was associated with pendulous udders (9.6% increase in SCC / cm drop) and greater total cross-sectional area of the teats (7.2% increase of SCC / cm^2). Higher HSCC was observed in older and thinner ewes. Lower HSCC was associated with heavier mean litter weight (6.7% decrease in SCC per kg). Linear, quadratic and cubic terms for days in lactation were also significant.

3.2 INTRODUCTION

Mastitis in sheep causes economic losses because of costs of treatment, ewe replacements, and reduced milk production (Albenzio *et al.*, 2002). In sheep industries where meat rather than milk is the predominant focus of production, a reduction in milk yield from mastitis is of commercial importance because of its negative effect on lamb growth rate. However, few studies have investigated the effect of intramammary infection on lamb growth and to date, there has been no comprehensive prospective longitudinal study of the impact of intramammary infection on lamb weight in suckler ewes that accounts for other udder health variables such as udder conformation, teat lesions and hygiene at lambing and factors that affect lamb growth. There were two aims of the current study. The first was to investigate the relationship between SCC in milk and lamb weight whilst adjusting for other factors that influence lamb weight. The second was to further our understanding of the factors associated with milk SCC of suckler ewes.

3.3 MATERIALS AND METHODS

3.3.1 Study farm and ewe selection

A farm in Shropshire, England was convenience selected on willingness to participate, management of ewes in separate age groups and handling facilities that enabled longitudinal observation of ewes and lambs. A total of 78 ewes were enrolled into the study in December 2009: the study group comprised 20 2- yr old Suffolk mules, 20 6 year old Suffolk mules and 38 8 year old North Country mules.

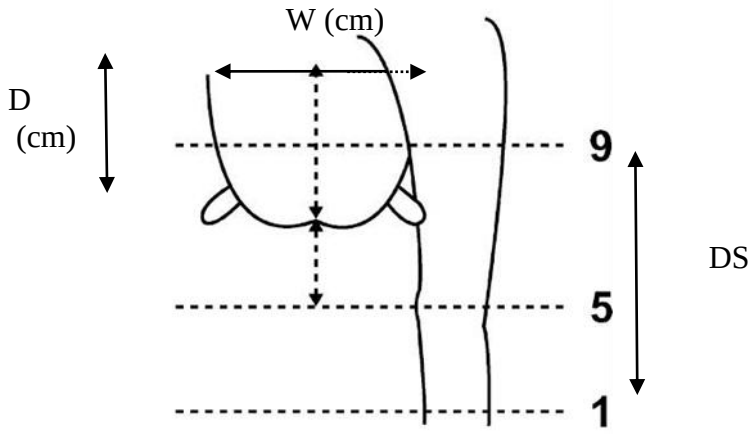
3.3.2 Collection of ewe and lamb data

In February 2010, one month before lambing was due to start, ewes selected for study were examined and their ear tag number, the presence of palpable intramammary lesions and body condition score (Defra PB1875, undated) were recorded. Within 12-72 hours of lambing, each ewe and litter was examined in an individual lambing pen. Each lamb was identified with an ear tag and all clinical abnormalities were recorded. Lambs were weighed using an ISO 9001:2008 assured hanging scale with 0.1kg calibrations (Salter 235-6S) and the sex and litter size recorded. The body condition score of each ewe was recorded. With the ewe in pelvic recumbency, the udder was examined and all visible and palpable abnormalities including scars on the udder and teats were recorded. Teat lesion type, depth, position and orientation were recorded and later classified as traumatic or non-traumatic. Traumatic teat lesions appeared acute in onset and included bite wounds, tears and chapping. Non-traumatic lesions included orf-like lesions, warts, spots and other proliferative lesions that appeared chronic. A milk sample was collected from each udder-half for somatic cell counting.

After lambing, ewes were managed in four groups categorised by age, and litter size. The groups were 2 and 6 year old Suffolk mules with single lambs, 2 and 6 year old

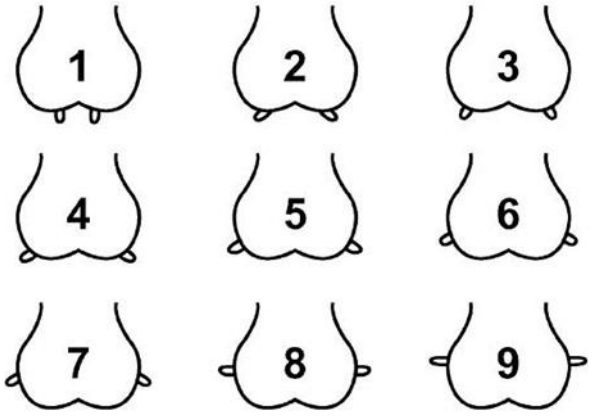
Suffolk mules with multiple lambs, 9 year old North Country mules with single lambs and 9 year old North Country mules with multiple lambs. Each group was brought in from the fields into a sheltered handling facility for examination. Ewes and lambs were examined every 14 days from lambing until lambs were eight to ten weeks old. On each occasion lambs were weighed in a calibrated weigh crate and ewes were cast in pelvic recumbency in a cradle. Ewes and lambs were examined and milk samples collected following the same protocols as at lambing. Fourteen days after lambing, detailed measurements of the udder were made and the udder conformation was scored using to a nine point scoring system developed by Casu *et al.* (2006) (Figure 3.1) with the ewe standing and then in pelvic recumbency.

Figure 3.1. **Udder conformation scoring and measurement chart**



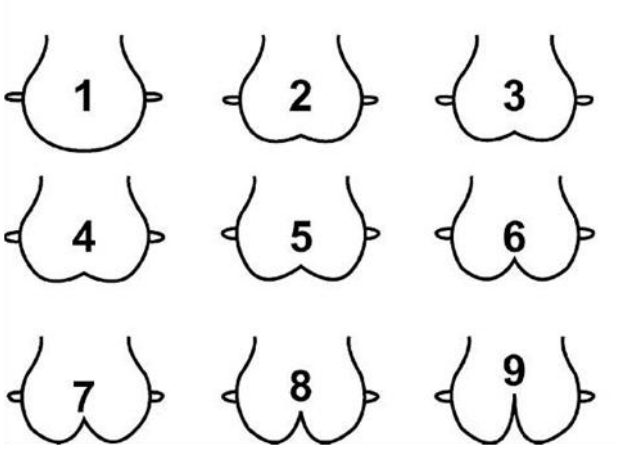
D: Udder drop as measurement (cm)
DS: Udder drop as score: 1 (nearest to ground) to 9 (furthest from ground) in standing ewe
W: Width of udder base as measurement (cm)

Figure 1. continued



TPS

TPS: Teat placement score: 1 (most medial) to 9 (most lateral)



SS

SS: Udder separation score: 1 (no separation) to 9 (maximum separation)

Figures adapted from Casu et al., 2006

3.3.3 Collection of milk samples

Milk samples for somatic cell counting were collected and processed as previously described in Chapter 2.

3.3.4 Data storage and analysis

A database was constructed in Microsoft Access 2007 into which observation date, ewe ID, body condition score, SCC, udder conformation scores and measurements and abnormalities of the udder, teat and milk were recorded. A second linked sheet

was used to store lamb ID, litter size, lamb weight and whether lambs were thin, had diarrhoea or had orf-like lesions on the muzzle.

Descriptive analysis was performed in Stata 10 (StatCorp LP, Texas). The somatic cell count data were $\log_{10}$ transformed and normality of each of the two outcome variables assessed using Q-Q plots. Strata were merged where adjacent categories had less than six observations. Repeated measures of SCC were plotted over weeks in lactation. Log_{10} mean somatic cell count for each ewe at each observation was categorised into quintiles to investigate the impact of SCC on lamb weight.

Mixed effect models were developed in MLwiN 2.11 (Rasbash *et al.*, 2005). Two three-level linear multivariable regression models were constructed; the first with lamb weight (kg) as the continuous outcome variable with ewe, lamb and observation as levels 3, 2 and 1 random effects; the second with $\log_{10}$ HSCC (cells/ml) as the continuous outcome variable with ewe, udder-half and observation as levels 3, 2 and 1 random effects. Each model took the general structure:

$$y_{ijk}=\beta_0+\beta x_{ijk}+\beta x_{jk}+\beta x_k+v_k+u_{jk}+e_{ijk}$$

where yijk was the continuous outcome variable and β_0 was a series of vectors of fixed effects that vary at levels *ijk* (observation), *jk* (lamb in model 1; udder half in model 2) and *k* (ewe), with variance estimates *v, u* and *e*. The independent variables were tested in the model using a manual forward stepwise selection process. Significance was set at 0.05. Where similar and highly correlated explanatory variables were tested and significant in the multivariable model, the variable that most improved the model fit to the data was retained.

3.4 RESULTS

3.4.1 Descriptive analysis

From the 78 ewes enrolled, 73 ewes lambed over a period of 49 days. Sixty-seven ewes that had at least one lamb that survived for a minimum of three observations and that yielded a milk sample for somatic cell counting on at least three occasions from a minimum of one udder-half were included in the analysis. Four ewes were lost to follow up due to death, including one ewe with acute clinical mastitis after lambing. A further two ewes were omitted from the analysis due to insufficient SCC or lamb weight data. One ewe developed acute clinical mastitis 45 days after lambing; data from this ewe and her lambs were included in the analysis until day 45. Of the 67 ewes that were included in the analysis, 35 reared one lamb, 31 reared twins and one reared triplets; two ewes had one foster lamb. Summary statistics for continuous variables are presented in Table 3.1 and for categorical variables in Table 3.2. Forty-nine ewes had at least one teat lesion. Younger ewes and ewes rearing one lamb had a higher BCS. Modal BCS was 3.5 for the group of 2 and 6 year old Suffolk mules rearing one lamb, 3 for the group of 2 and 6 year olds Suffolk mules rearing more than one lamb, 2.5 for the group of 9 year old North Country mules rearing one lamb and 2 to 2.5 for 9 year old North Country mules rearing more than one lamb. Change in modal BCS by group from before lambing to the end of the observation period was slight, with at most a body condition score change of 0.5 over the study.

There were 101 lambs that were followed. Twins and triplets were combined as multiples. Fifty-nine lambs were male and 42 female, 19 lambs had orf-like lesions, which were observed on the lambs' muzzles, 39 lambs had diarrhoea and 33 were visibly thin on at least one occasion.

Table 3.1. Summary statistics and observations for continuous variables

Continuous variables	Min	Max	Mean	Std. Dev.	n observations
Lamb age (days)	0	102	38.12	27.95	592
Birth weight (kg)	2.30	8.4	5.25	1.25	101
Biweekly lamb weight (kg)	2.30	36.9	13.16	6.83	592
Log SCC left udder-half	4.45	7.34	5.38	0.52	278
Log SCC right udder-half	4.53	7.65	5.52	0.64	290
Log SCC both udder-halves	4.45	7.65	5.45	0.59	568
Number of days ewe fed concentrates before lambing	37	85	61.66	9.68	67
Number of days BCS recorded before lambing	8	56	32.66	9.68	67
Drop of udder (cm)	11.40	24.10	16.83	2.75	64
Width at base of udder (cm)	7.90	23.0	17.26	2.77	65
Left teat length (cm)	2.50	5.00	3.38	0.56	66
Right teat length (cm)	2.50	5.10	3.55	0.58	66
Left teat width (cm)	1.00	2.50	2.07	0.34	66
Right teat width (cm)	1.00	3.0	2.05	0.43	66
Sum cross sectional area of both teats (cm^2)	7.50	15.00	11.06	1.50	66

Table 3.2. Summary statistics and observations for categorical variables

Categorical variables	n observations	N	% of observations
Ewe age (at lambing)			
2yr	19	67	28.36
6yr	19	67	28.36
9yr	29	67	43.28
Litter size			
one lamb	35	67	52.24
two lambs	31	67	46.27
three lambs	1	67	0.15
Teat placement scores			
1 - 3 (most medial)	12	64	18.75
4	13	64	21.88
5	14	64	20.31
6	12	64	18.75
7 - 9 (most lateral)	13	64	20.31
Degree of udder separation (score)			
1 (minimum separation)	22	64	34.38
2	20	64	31.25
3	14	64	21.88
4 - 9 (maximum separation)	8	64	12.50
Udder drop score in standing ewe			
1 (greatest depth) to 5	17	65	26.15
6	24	65	36.92
7 to 9 (least depth)	24	65	36.92
Wool on udder			
No	53	66	80.30
Yes	13	66	19.70
Cleanliness of bedding at lambing			
Clean	30	65	46.18
Moderately dirty	17	65	26.15
Very dirty	18	65	27.69
Water availability at lambing			
Unrestricted	20	65	30.77
Restricted	27	65	41.54
No water available	18	65	27.69
BCS before lambing (4 categories)			
2 or less	8	67	11.94
2.5	24	67	35.82
3	20	67	29.85
3.5 or more	15	67	22.39
BCS at biweekly observation			
1.5 or less	24	401	0.06
2	70	401	0.17
2.5	97	401	0.24
3	120	401	0.30
3.5	56	401	0.14

3.5 or more	34	401	0.08
Ewe had either a traumatic or a non-traumatic teat lesion on at least one teat at any point through entire study period	49	67	73.13
Teat had either a traumatic or a non-traumatic teat lesion at any point through entire study period	87	125	69.60
Teat had a traumatic teat lesion on at any point during the entire study period	67	125	53.60
Teat had a non-traumatic teat lesion at any point during the entire study period	55	125	44.00
Traumatic lesion (bites, tears, chapping) observed on either teat at visit	87	566	15.37
Non traumatic lesion (orf, warts, spots) observed on either teat at visit	51	566	9.01
Lesion at or near teat orifice observed at visit	163	568	28.70
Pustule or papule on teat observed on teat at visit	31	568	5.46
Lamb had diarrhoea	39	591	6.60
Lamb had orf	19	592	3.21
Lamb visibly or palpably thin	33	591	5.58
Udder contaminated with faeces or mud at visit	29	401	6.25

There were 592 observations of 101 lambs between birth and 15 weeks of age. At the first observation of lambs their mean age was 1.6 days and the mean weight was 5.3 kg. Mean lamb weight increased with age although variability in lamb weight was similar across all weeks of age up until 13 weeks where after there were observations from only two lambs (Table 3.3).

Table 3.3. Lamb weight by age of lamb in weeks

Lamb weight (kg)						
Wk	**Mean**	**95% Confidence Interval**		**Median**	**Coeff. Var**	**n**
		Lower	**Upper**			
1	5.26	2.85	7.67	5.3	4.3	103
2	8.01	4.39	11.64	7.8	4.3	59
3	8.84	4.06	13.62	8.4	3.6	44
4	11.09	6.34	15.84	10.9	4.6	49
5	11.61	5.47	17.75	10.95	3.7	44
6	13.85	7.02	20.69	12.95	4.0	44
7	14.06	5.60	22.53	13.3	3.3	31
8	16.99	7.99	25.99	16.25	3.7	38
9	18.83	10.25	27.42	18.8	4.3	24
10	19.61	8.77	30.45	18.7	3.6	57
11	19.41	9.57	29.25	18.3	3.9	34
12	22.47	9.71	35.32	22.1	3.5	46
13	20.12	10.24	29.99	19.9	4.0	17
15	19.00	14.01	23.99	19.00	7.5	2
Total	13.16	-0.22	26.46	22.1	1.9	592

There were 568 HSCC measurements from 67 ewes. The $\log_{10}$ HSCC ranged from 4.45 to 7.65 with a mean $\log_{10}$ SCC over all observations of 5.45, a geometric mean SCC of 281 x 10^3 cells/ml. The arithmetic mean SCC was 1,119 x 10^3 cells/ml.

The maximum and minimum values of quintiles of mean ewe SCC at each observation are given in Table 3.4.

Table 3.4. Quintiles of mean ewe SCC and mean ewe $\log_{10}$ SCC

	Min ewe SCC in quintile		**Mean ewe SCC in quintile**		**Max ewe SCC in quintile**	
Quintile	cells/ml	$\log_{10}$ cells/ml	cells/ml	$\log_{10}$ cells/ml	cells/ml	$\log_{10}$ cells/ml
1	37417	4.57	73859	4.87	115724	5.06
2	115931	5.06	148197	5.17	199158	5.30
3	202228	5.31	280336	5.45	391029	5.59
4	391497	5.59	497368	5.70	692503	5.84
5	697676	5.84	1565548	6.19	10781510	7.03

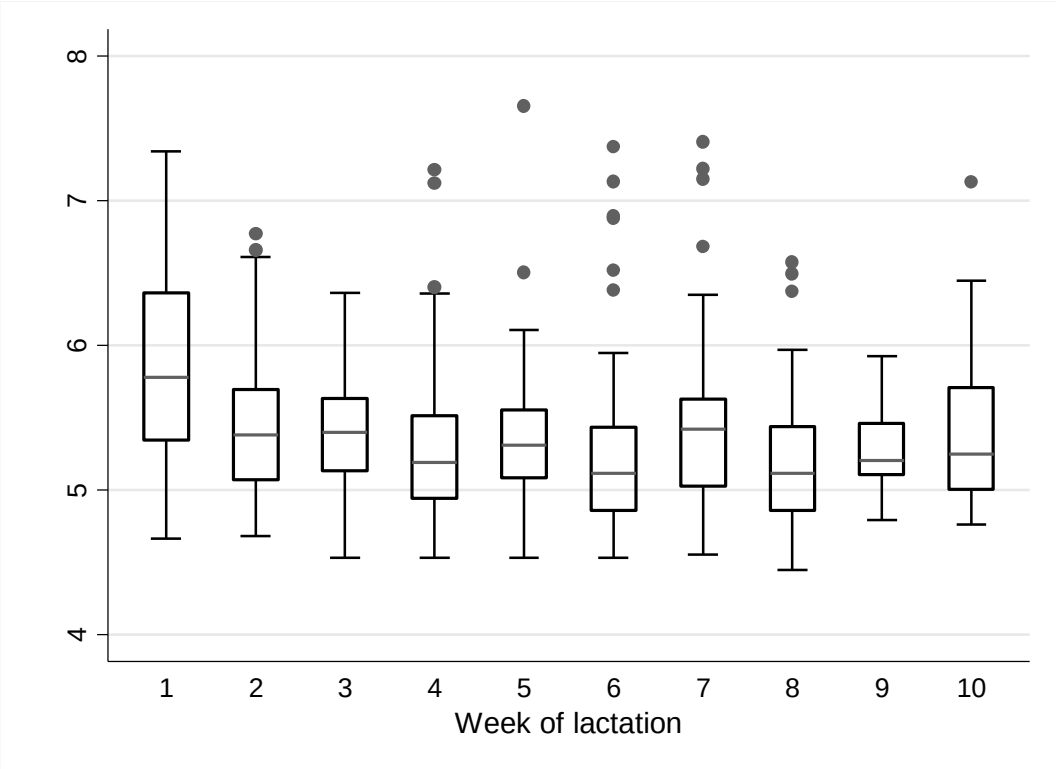
The mean log_{10} SCC was significantly higher in the first week after lambing compared with subsequent weeks ($p<0.05$) with a general pattern of decreasing SCC in the first four weeks of lactation followed by a trend of gradual increase trend five to ten weeks post lambing and a small peak at around seven weeks in lactation (Table 3.5 and Figure 3.2).

Variability in SCC was not constant across all weeks (Table 3.5); the coefficient of variation was higher in weeks 3 and 9 of lactation, although there were smaller numbers of observations in these weeks.

Table 3.5. Log_{10} SCC measurements by weeks in lactation

	log_{10}SCC					
Wk	**Mean**	**95% Confidence Interval**		**Median**	**Coeff. Var**	**n**
		Lower	**Upper**			
1	5.88	4.60	7.16	5.78	9.0	106
2	5.45	4.45	6.46	5.38	10.6	64
3	5.4	4.60	6.21	5.4	13.2	54
4	5.33	4.28	6.38	5.19	10.0	62
5	5.36	4.38	6.34	5.31	10.7	59
6	5.28	4.00	6.55	5.11	8.1	57
7	5.49	4.13	6.84	5.42	7.9	40
8	5.19	4.30	6.08	5.11	11.5	53
9	5.29	4.79	5.78	5.2	21.0	38
10	5.43	4.38	6.48	5.25	10.1	35
Total	5.45	4.30	6.60	5.32	9.3	568

Figure 3.2 Box and whisker plot of log_{10}SCC over weeks in lactation



Log_{10} SCC in left and right udder halves was highly correlated (r = 0.87). Ewe age was positively correlated with breed (r = 0.82), and negatively correlated with BCS (r = -0.62), consequently BCS and breed were negatively correlated (r = -0.64). A teat lesion at or near a teat orifice had a low correlation with other variables including days in lactation (DIL) (r = 0.15). A list of all variables assessed in univariable analysis of the continuous outcomes of lamb weight (kg) and log_{10} SCC that were not in the final multivariable models are presented in Table 3.6 and Table 3.8 respectively. Correlations of variables are presented in Table 3.10 and Table 3.11.

Table 3.6. Univariable coefficients of variables not included in final multivariable model of lamb weight

Variable	Coefficient	95% Confidence Interval	
		lower	upper
Response Variable is lamb weight (kg) (n=592)			
(Lamb age (days))^2*	2.30 x 10^{-3}	2.20 x 10^{-3}	2.40 x 10^{-3}
(Lamb age (days))^3*	2.58 x 10^{-5}	2.43 x 10^{-5}	2.72 x 10^{-5}
Lamb age in weeks*	1.48	1.43	1.53
Days of concentrate feed before lambing	0.01	-0.07	0.10
Drop of udder (cm)	-0.12	-0.40	0.16
Width of base of udder (cm)	0.03	-0.24	0.30
Average teat length (cm)	-0.72	-2.25	0.82
Total teat length (cm)	-0.36	-1.13	0.41
Average teat width (cm)	-0.11	-2.35	2.13
Total teat width (cm)	-0.06	-1.17	1.06
Average cross sectional area of both teats (cm^2)	-0.35	-1.38	0.69
Total cross-sectional area of both teats	-0.02	-0.12	0.08
Log SCC left udder-half*	-3.95	-4.99	-2.90
Log SCC right udder-half*	-3.53	-4.41	-2.65
Mean log SCC*	-5.24	-6.36	-4.12
Max Log SCC*	-3.40	-4.25	-2.55
(Mean Log SCC)^2*	-1.01	-1.23	-0.80
(Max Log SCC)^2*	-0.55	-0.69	-0.40
Max Log SCC categorised into quintiles*			
1	Reference		
2	-2.17	-3.65	-0.68
3	-2.61	-4.14	-1.07
4	-5.14	-6.70	-3.58
5	-6.14	-7.78	-4.50
Sex of lamb			
male	Reference		
female	-0.85	-2.13	0.43
Lamb has suspected orf*	5.19	2.17	8.22
Ewe age			
2yr	-0.59	-2.51	1.33
6yr	Reference		
9yr	-1.87	-3.61	-0.12
Breed of ewe *			
Suffolk mule	Reference		
North of England mule	-1.60	-3.11	0.09
Visit BCS *			

1.5 or less	Reference		
2	3.06	0.55	5.56
2.5	1.90	-0.63	4.44
3	5.47	2.96	7.99
3.5 or more	2.56	-0.12	5.24
Udder separation score			
1 (minimum)	-1.75	-6.38	2.87
2	-3.07	-7.71	1.56
3	-1.08	-5.79	3.63
4	Reference		
5	1.91	-3.63	7.44
6	-0.89	-6.59	4.81
7	-1.85	-9.12	5.43
8 to 9 (maximum separation)	No observations		
Udder depth score			
1 (maximum depth) to 5	Reference		
6	0.18	-1.74	2.10
7 to 9 (minimum depth)	-0.09	-2.03	1.86
1 (most medial) to 3	-0.21	-2.70	2.29
4	0.20	-2.19	2.59
5	ref		
6	-0.82	-3.27	1.63
7 to 9 (most lateral)	0.22	-2.15	2.59
1 (most medial) to 3	-0.21	-2.70	2.29
Udder contamination observed at visit	-0.85	-3.10	1.41
Udder contamination observed at the previous visit	1.43	-0.79	3.64
Wool on udder	-0.85	-2.65	0.96
Teat placement scores			
1 (most medial) to 3	-0.21	-2.70	2.29
4	0.20	-2.19	2.59
5	ref		
6	-0.82	-3.27	1.63
7 to 9 (most lateral)	0.22	-2.15	2.59
Teat placement scores			
Bedding at lambing			
Clean	Reference		
moderately dirty	1.49	-0.35	3.32
very dirty	-0.54	-2.32	1.23
Water availability at lambing			
unrestricted	Reference		
Restricted	-0.39	-2.15	1.36
no water available	0.89	-1.14	2.92
Teat lesion of any type observed on either teat at visit*	2.95	1.89	4.00

Traumatic teat lesion observed on either teat at visit*	1.92	0.73	3.11
Non-traumatic teat lesion on either teat observed on the previous visit*	3.45	2.03	4.87
Teat lesion of any type observed on either teat at the previous visit*	3.25	2.13	4.37
Ewe had a teat lesion of any type on either teat through entire study period	-0.75	-2.50	1.00
Ewe had a traumatic teat lesion on either teat at any point in the study period*	-1.53	-3.04	-0.03
Ewe had a non-traumatic teat lesion on either teat at any point in the study period	-1.32	-2.82	0.18

***** denotes was significant in univariable analysis, but not included in multivariable lamb weight model

Approximately equal numbers of ewes had teat placement scores of 4, 5, 6 and 7 (mode of 5, n=14) with relatively few ewes with a teat placement of 2, 3 or 8. No ewes were classified as having teat placement scores of 1 or 9.

The interquartile range of udder drop measurements was between 15.2cm and 19cm. Older ewes tended to have more pendulous udders; the majority of 6 and 9 year old ewes had an udder drop score of 6 whilst most 2 year old ewes had an udder drop score of 7, where a lower score indicates greater drop. The greatest udder drop score was 5 and approximately a third of 9 year old ewes had this score whilst only one 2 year old ewe had this score.

There were few observations (six ewes) of teat lesions in the first three weeks after lambing. The peak incidence of traumatic teat lesions occurred three to four weeks after lambing, in 30% of ewes. Thereafter the incidence decreased gradually until nine to ten weeks after lambing when 10% of ewes had a new teat lesion. The incidence of non-traumatic lesions was also higher three to four weeks after lambing (8% of ewes) than in previous weeks. Non-traumatic teat lesion incidence gradually increased until week 9-10 after lambing (16% of ewes).

3.4.2 Multivariable analysis of lamb weight

Significantly lower lamb weights were associated with a ewe SCC > 400,000 cells/ml and a traumatic teat lesion 14 days previously. Teat lesions were associated with a decrease in lamb weight; a traumatic teat lesion at the previous visit was significantly associated with lambs weighing 0.65kg less compared with lambs reared by a ewe where a traumatic teat lesion had not been observed. Lambs reared by ewes with a mean SCC > 400,000 cells/ml weighed 1.70kg less at each observation. Lamb age, birth weight, litter size, presence of diarrhoea and ewe BCS before lambing were also associated with lamb weight. A non traumatic teat lesion was associated with a lower lamb weight (-1.1.kg) significant at 90% confidence level (Table 3.7).

The association of lamb weight with higher ewe SCC was demonstrated most clearly by separating $\log_{10}$ ewe SCC into quintiles. There was an overall trend that the higher the SCC the lower the lamb weight, with ewe SCC > 116,000 cells/ml, and SCC between 116,000 cells/ml and 202,000 cells/ml significantly associated with a 0.58kg lower lamb weight. Ewe somatic cell counts in the third quintile, between 202,000 cells/ml and 391,000cells/ml, were also associated with lower lamb weight (0.31kg) this was only significant at the 90% confidence level. However, ewe SCC in the top two quintiles, >391,000 cells/ml were significantly associated with lambs weighing 1.70 kg less for a ewe mean SCC of >391,000 cells/ml or 1.39kg less for a ewe SCC > 698, 000 cells/ml.

Lambs reared as multiples weighed 1.67 kg less at each observation than those reared as single lambs. Lambs with diarrhoea weighed 1.19kg less than lambs without diarrhoea. There was a prevalence of 72.2% of diarrhoea in lambs in the group of 9 year old North Country mules rearing singles whilst there was zero prevalence of

diarrhoea in lambs in the group of 9 year old North Country mules with twins. Ewe body condition score before lambing was significantly associated with a difference in lamb weight; lambs reared by ewes that were in BCS of 2.5 or less before lambing were significantly lighter than those reared by ewes in higher body condition score weighing on average 1.29kg less.

The observation of an orf-like lesion on a lamb was not associated with a change in lamb weight. Hygiene of the pen or udder and the availability of water to the ewe at lambing were not significantly associated with lamb weight.

Residual variances were significant at the ewe and visit level, indicating that some of the between ewe and between visit variance remained unexplained. At the lamb level, residual variance was low and non-significant, thus variation between lambs was explained by the variables in the model.

Residuals and standardised residuals were normally distributed and the model provided a good fit to the data (-2 x log likelihood = 1313; Table 3.7) although some visits provided relatively high leverage in the model (Figure A.3.1).

Table 3.7. Multivariable model of lamb weight including univariable values for those variables significant in the multivariable analysis.

Variable	Univariable coefficient	95% CI		Multivariable coefficient	95% CI	
		lower	upper		lower	upper
Response variable is lamb weight (kg)						
Intercept	13.41	12.64	14.12	1.45	-1.26	4.15
Lamb age (days)	0.21	0.20	0.21	0.22	0.20	0.23
Birth weight (kg)	1.91	1.50	2.32	1.56	1.24	1.88
one lamb	Reference			Reference		
two or more lambs	-3.70	-4.73	-2.67	-1.67	-2.51	-0.83
Lamb has diarrhoea	4.11	1.94	6.28	-1.15	-1.88	-0.43
Mean Log SCC						
1st quintile	Reference			Reference		
2nd quintile	-1.09	-2.62	0.45	-0.58	-1.13	-0.02
3rd quintile	-2.03	-3.58	-0.49	-0.31	-1.91	0.29
4th quintile	-4.03	-5.58	-2.47	-1.70	-2.36	-1.04
5th quintile	-6.70	-8.30	-5.08	-1.39	-2.16	-0.62
Ewe BCS before lambing						
3 or more	Reference					
2.5 or less	-1.04	-2.56	0.48	-1.29	-2.06	-0.52
Non traumatic lesion on either teat at visit	3.27	1.93	4.61	-1.08	-1.57	0.58
Traumatic lesion on either teat at previous visit	2.33	1.07	3.60	-0.65	-1.18	-0.12
	Variance	**95% CI**		**Variance**	**95% CI**	
		Lower	**Upper**		**Lower**	**Upper**
Between ewe residual variance	5.11	1.68	8.55	1.64	0.73	2.55
Between lamb residual variance	0.00	0.00	0.00	0.48	-0.08	1.02
Between visit residual variance	41.77	36.73	46.81	2.10	1.71	2.48
				-2 x log likelihood=1313.631 (312 out of 592 cases used)		

3.4.3 Multivariable analysis of somatic cell count

Variables assessed in the univariable analysis of $\log_{10}$ HSCC as the continuous dependent variable, with ewe, udder half and visit as random levels are listed in Table 3.8.

Significantly higher udder half somatic cell counts were seen in old and thin ewes, those with a greater depth of the suspended udder, and those with larger teat cross-sectional area. Other significant variables accounted for in the model were days in lactation and average lamb weight at that observation of lambs being reared by that ewe (Table 3.9).

Table 3.8. Univariable coefficients of variables not included in multivariable model of log_{10} HSCC

Variable	Coefficient	95% CI	
		Lower	Upper
Response variable is log_{10} udder half somatic cell count (n=568)			
Weeks in lactation*	-0.05	-0.06	-0.04
Total birth weight (kg)	0.03	-0.01	0.07
Average birth weight (kg)	0.00	-0.07	0.07
Total lamb weight at observation (kg)*	-0.02	-0.03	-0.01
Average lamb daily live weight gain (kg/day)*	-0.52	-0.94	-0.10
Average lamb daily live weight gain^2 (kg/day)^2	-0.73	-1.66	0.21
Left teat length (cm)	0.11	-0.05	0.27
Left teat width (cm)	0.25	-0.01	0.52
Left teat length x width (cm^2)	0.04	-0.00	0.08
Right teat length (cm)*	0.20	0.04	0.36
Right teat width (cm)*	0.24	0.03	0.45
Right teat length x width (cm^2)*	-0.06	-0.09	-0.02
Average teat length (cm)*	0.20	0.02	0.38
Total teat length cm)*	0.10	0.01	0.19
Average teat width (cm)*	0.31	0.05	0.56
Total teat width (cm)*	0.15	0.02	0.28
Average cross-sectional area of both teats (cm^2)*	0.06	0.02	0.10
Width of base of udder in standing ewe (cm)	0.02	-0.01	0.05
Udder drop as measurement x width of udder base as measurement (cms^2)	0.00	0.00	0.00
Udder drop (cm) / udder width (cm)	0.27	-0.18	0.71
Udder drop (cm) + udder width (cm)*	0.03	0.01	0.05
Breed or ewe is North of England mule (Suffolk mule as ref)*	0.27	0.09	0.44
Litter size of 2 or more lambs	0.07	-0.11	0.25
Diarrhoea in at least one lamb*	-0.17	-0.32	-0.02
Orf in at least one lamb	-0.18	-0.38	0.03
At least one is lamb visibly or palpably thin	-0.01	-0.18	0.17
Udder separation score*			
1 (minimum separation)	Ref		
2	-0.15	-0.37	0.08
3	-0.06	-0.31	0.18
4 to 9 (maximum separation)	-0.37	-0.67	-0.08
1 (maximum depth) to 5	Reference		
6	-0.23	-0.44	-0.01
7 to 9 (minimum depth)	-0.32	-0.54	-0.11
BCS before lambing *			
2 or less	Reference		

2.5	-0.37	-0.67	-0.07
3	-0.41	-0.71	-0.11
3.5 or more	-0.52	-0.83	-0.20
Visit BCS*			
1.5 or less	Reference		
2	-0.16	-0.39	0.08
2.5	-0.27	-0.51	-0.03
3	-0.43	-0.68	-0.19
3.5 or more	-0.37	-0.62	-0.11
Teat placement score			
1 (most medial) to 3	0.08	-0.16	0.33
4 to 6	Reference		
7 to 9 (most lateral)	0.25	0.02	0.49
Traumatic or non traumatic teat lesion observed on teat at visit *	-0.15	-0.25	-0.04
Traumatic teat lesion observed on teat at visit*	-0.14	-0.25	-0.02
Traumatic teat lesion observed on teat at previous visit	-0.04	-0.15	0.07
Non-traumatic teat lesion observed on teat at visit*	-0.14	-0.29	-0.00
Non-traumatic teat lesion observed on teat at previous visit*	0.11	-0.03	0.26
Pustule or papule on teat observed on teat at visit	-0.09	-0.27	0.08
Pustule or papule on teat observed on teat at previous visit	-0.03	-0.19	0.13
Lesion at or near teat orifice observed at previous visit	-0.07	-0.15	0.01
Ewe had a teat lesion of either type on teat at any point through entire study period	-0.02	-0.22	0.18
Ewe had a traumatic teat lesion on teat at any point during the entire study period	0.02	-0.17	0.20
Ewe had a non-traumatic teat lesion on teat at any point during the entire study period	0.01	-0.17	0.19
Udder contamination observed at visit	-0.11	-0.25	0.03
Udder contamination observed at the previous visit	-0.08	-0.21	0.06
Woolly udder	0.01	-0.23	0.24
Bedding at lambing			
clean	Reference		
moderately dirty	0.11	-0.10	0.33
very dirty	0.13	-0.09	0.35
Water availability at lambing			
unrestricted	Reference		
restricted	-0.02	-0.24	0.20
no water available	-0.02	-0.26	0.23

* denotes was significant in univariable analysis, but not included in final $\log_{10}$ HSCC model

The older the ewe, the less the relative effect of body condition score on HSCC. Nine year old ewes that were less than BCS 3 during lactation had a significantly higher HSCC than the reference category of 2 year old ewes that were in BCS 2, indeed, those 9 year olds of BCS of 1.5 or less had a HSCC that was 86% higher than 2 year old ewes in BCS 2. Six year old ewes that were less than BCS 2 during lactation also had significantly higher HSCC than the reference category, showing a five-fold increase in SCC.

A greater udder drop measurement was associated with higher HSCC, with a 10% increase in SCC for every centimetre increase in udder drop. Larger teats were also associated with higher HSCC, with a 7% increase in HSCC for every $1cm^2$ increase in total cross-sectional area of teat.

The observation of an orf-like lesion on the teat or lamb or traumatic or non traumatic teat lesions on the teat was not associated with HSCC. Other variables not significantly associated with a change in HSCC were bedding cleanliness at lambing, availability of water to the ewe at lambing, and contamination of the udder.

Residuals and standardised residuals were normally distributed and the model provided a good fit to the data (Figure A.3.2). Residual variances at each level were low and only significant at the udder half and visit level. The majority of between udder half and visit variance and almost all of the variation between ewes was explained by the variables in the model.

Table 3.9. Multivariable model of log_{10} HSCC

Variable	Univariable coefficient	95% CIs		Multivariable coefficient	95% CIs	
		lower	upper		lower	upper
Response variable is log_{10} HSCC						
Intercept	5.48	5.39	5.57	4.85	4.29	5.42
Days in lactation	-0.01	-0.01	-0.01	-0.03	-0.05	-0.02
Days in lactation^2	-7.08 x 10^{-5}	-9.68 x 10^{-5}	-4.48 x 10^{-5}	9.31 x 10^{-4}	4.57 x 10^{-4}	1.41 x 10^{-3}
Days in lactation ^{3}	-8.30 x 10^{-7}	-1.24 x 10^{-6}	-4.20 x 10^{-7}	-6.74 x 10^{-6}	-1.52 x 10^{-5}	-1.96 x 10^{-6}
Average litter weight at observation (kg)	-0.03	-0.04	-0.03	-0.03	-0.05	-0.01
Drop of udder (cm)	0.06	0.03	0.09	0.04	0.01	0.07
Total cross sectional area of both teats (cm^2)	0.03	0.01	0.05	0.03	0.01	0.05
Lesion at or near teat orifice observed at visit	-0.20	-0.29	-0.11	-0.11	-0.19	-0.03
2 year old ewe, BCS of 3 or more	Reference			Reference		
6 year old ewe, BCS of 3	0.09	-0.09	0.26	0.08	-0.08	0.24
6 year old ewe, BCS of 2.5	0.10	-0.11	0.32	0.08	-0.12	0.29
6 year old ewe, BCS of 2	0.27	-0.12	0.65	0.35	-0.08	0.78
6 year old ewe, BCS of 1.5 or less	0.94	0.41	1.48	0.70	0.23	1.17
9 year old ewe, BCS of 3	0.14	-0.17	0.45	0.12	-0.15	0.39
9 year old ewe, BCS of 2.5	0.24	0.05	0.44	0.19	0.00	0.37
9 year old ewe, BCS of 2	0.30	0.11	0.49	0.20	0.01	0.38
9 year old ewe, BCS of 1.5 or less	0.34	0.06	0.62	0.27	0.02	0.52
	Variance	**95% CI**		**Variance**	**95% CI**	
		lower	**upper**		**lower**	**upper**
Between ewe residual variance	0.07	0.02	0.13	0.02	-0.02	0.06
Between udder-half residual variance	0.09	0.04	0.14	0.11	0.06	0.15
Between visit residual variance	0.19	0.16	0.21	0.13	0.12	0.15
-2 x log likelihood=646.116 (539 out of 568 cases)						

Table 3.10. Correlations of >0.5 of explanatory variables in multivariable models

Variable	Correlated variables (Correlation coefficient)
Lamb age (days) (or days in lactation)	Non traumatic lesion (orf, warts, spots) observed on either teat at visit (-0.64*), traumatic lesion (bites, tears, chapping) observed on either teat at visit (-0.64*)
Birth weight (kg)	Litter size (multiple lamb litter) (-0.57*)
Number of days ewe fed concentrate before lambing (earliest lambing ewe as reference)	*No correlations over 0.50*
Drop of udder with ewe standing (cm)	Udder drop of with ewe standing as score (0.80*), Udder width at base with ewe standing (cm) (0.66*)
Total cross sectional area of both teats (cm^2)	Udder drop of with ewe standing as score (0.68*) or as measurement (cm) (0.55*), teat placement (0.55*), separation of udder halves (0.55*), udder width at base with ewe standing (cm) (0.72*)
Ewe body condition score	Breed of ewe (0.82*), ewe body condition score before lambing (0.59*)
Ewe age	BCS at visit (0.63*), Breed of ewe (0.82*)
Sex of lamb[$]	*No correlations over 0.50*
Litter size (multiple lamb litter)	*No correlations over 0.50 (except birth weight -0.57*)*
Lamb has diarrhoea	*No correlations over 0.50 (except birth weight -0.57*)*
Mean log SCC (quintiles)	Max logSCC (1.00*), Non traumatic lesion (orf, warts, spots) observed on either teat at visit (-0.64*), traumatic lesion (bites, tears, chapping) observed on either teat at visit (-0.64*). Length of teat (cm) (0.77*)
Teat placement scores (1(most medial) to 3, 4, 5, 6, 7 to 9 (most lateral))	Udder drop of with ewe standing as score (1.00*) or as measurement (cm) (0.89*), Udder width at base with ewe standing (cm) (0.66*). Degree of separation of udder halves as score (1.00*)
Lesion at or near teat orifice observed at visit	Average daily live weight gain (0.99*), lamb age (days) (0.64*), average lamb weight (0.57*)
Non traumatic lesion (orf, warts, spots) observed on either teat at visit	Days in lactation (-0.64*), Average daily live weight gain (0.99*)
Traumatic lesion (bites, tears, chapping) observed on either teat at previous visit	*No correlations over 0.50*

* value significant at 95% confidence level

Table 3.11. Correlations of explanatory variables in multivariable models

	Lamb age (days)	Birth weight (kg)	Days of concentrate feed	Udder Drop (cm)	Total cross sectional area of both teats (cm^2)	Ewe BCS	Ewe age (yrs)	Sex of lamb[$]	Litter size	Lamb had diarrhoea	Mean Log SCC (quintiles)	Teat placement scores	Lesion at or near teat orifice at visit	Non traumatic lesion on either teat at visit	Traumatic lesion on either teat at previous visit
Lamb age (days)	1														
Birth weight (kg)	-0.02	1													
Days of concentrate feed	-0.12*	-0.26*	1												
Udder Drop (cm)	-0.01	0.16*	-0.02	1											
Total cross sectional area of both teats (cm^2)	-0.02	0.31	0.03	0.77*	1										
Ewe BCS	-0.03	-0.03	-0.04	0.01	0.10*	1									
Ewe age (yrs)	-9×10^{-4}	0.22*	-9×10^{-4}	0.07	4.7×10^{-3}	-0.62*	1								
Sex of lamb[$]	-0.02	-0.28*	0.05	0.10	0.03	0.15*	-0.15*	1							
Litter size	0.03	-0.58	-0.24*	-0.24*	-0.44*	-0.31*	0.11*	-4 x 10-^{3}	1						
Lamb had diarrhoea	-2×10^{-4}	0.03	0.02	-0.01	-7.3×10^{-3}	0.01	1×10^{-3}	-0.05	-0.03	1					
Mean Log SCC (quintiles)	-0.54*	-0.02	0.17*	0.18*	-0.04	0.08	-0.01	0.03	0.08	-0.03	1				
Teat placement scores	-0.01	0.16	-0.02	1.00*	0.77*	0.01	0.07	0.01*	-0.24*	9.5×10^{-3}	-0.04	1			
Lesion at or near teat orifice observed at visit	0.15*	0.07	-	0.04	0.02	0.07	0.09*	-	0.02		-0.04	0.04	1		
Non traumatic lesion observed on either teat at visit	-0.64*	-0.04	-0.16*	-0.04	-0.03	0.06	-0.03	0.03	0.07	-0.02	0.91*	-0.04	0.04	1	
Traumatic lesion observed on either teat at previous visit	0.31*	-0.05	0.12*	-0.06	-0.03	-0.01	0.01	0.03	0.13	-0.03	0.17*	-0.06	-	0.11*	1

3.5 DISCUSSION

This is the first study to simultaneously investigate the relationships between udder conformation, teat damage, SCC and lamb weight in suckler ewes. An association between udder conformation and higher HSCC was demonstrated, suggesting that ewes with poor udder conformation are at a higher risk of udder infection. Ewes with somatic cell counts of above 400,000 cells/ ml and teat damage reared significantly lighter lambs. An association of lower lamb weights with subclinical mastitis has previously been demonstrated but this is the first study to show that lamb weights have a relationship with other aspects of udder health. The level of ewe SCC above which lambs weigh less, having controlled for other factors affecting lamb weight, suggests a level at which functional impairment of the udder resulting in lower milk production occurs and such a level may be considered indicative of subclinical mastitis. Furthermore, lamb weights and SCC were both associated with ewe BCS indicating that appropriate nutrition of the ewe is of importance for udder health and lamb production.

3.5.1 Somatic cell count

An association between subclinical mastitis and lower lamb weight was previously demonstrated; in an experimental longitudinal study, Fthenakis and Jones (1990) observed significantly lower growth rates and weight to 52 days of age in lambs reared by ewes with experimentally induced subclinical *Staphylococcus simulans* udder infection, compared with lambs reared by unchallenged ewes. A Canadian field study demonstrated a significantly lower weaning weight in lambs reared by ewes with subclinical mastitis than ewes without, having adjusting for lamb birth weight, litter size at lambing, sex of lamb and age at weaning. However ewes were only assessed at one time point (around 52 days in lactation) and CMT was used

rather than a continuous SCC measure (Arsenault *et al.*, 2008). High SCCs in dairy sheep have been associated with a decrease in milk production (Saratsis *et al.*, 1999; Gonzalo *et al.*, 2002) and a significant decrease in milk yield has been demonstrated in dairy cows with a mean SCC across all quarters of >200,000 cells/ml, even when adjusting for the dilution effects of milk yield on SCC (Green *et al.*, 2006). Thus it follows that lambs reared by ewes with higher SCC levels may have less milk available to them for growth.

3.5.2 Teat lesions

Traumatic teat lesions were significantly associated with lower lamb weight the following fortnight. A fresh traumatic teat lesion such as a bite wound or skin tear is likely to cause the ewe discomfort or pain when a lamb attempts to suckle and affect her compliance in allowing a lamb to feed thus resulting in lower lamb weight at the next observation. The discomfort experienced by the ewe by a lamb suckling is likely to be influenced by the nature and chronicity of the teat damage. Non traumatic teat lesions were associated with a lower lamb weight at that observation although this was only significant at the 90% level. Non-traumatic teat lesions were characterised by lesions that were more chronic in nature with proliferative scarring. Teat lesions of different types may interrupt milk flow by adversely affecting the efficiency of the mechanics of lamb suckling. The sudden increase and peak in incidence of traumatic teat lesions observed 3-4 weeks after lambing coincides with the eruption of lamb lower incisors. That the incidence of traumatic teat lesions decreased thereafter whilst there was an observed increase in non-traumatic teat lesion incidence in subsequent weeks is probably due to the healing process of traumatic teat lesions and any consequential scarring would have been recorded as a new observation of a non-traumatic teat lesion.

Teat lesions of either type were not significantly associated with a change in HSCC. This is in concordance of a different study where there was no significant association between the presence of teat lesions and subclinical mastitis, where a ewe was categorised as having subclinical mastitis if at least one of her udder halves was bacteriologically and WT positive (Watkins *et al.*, 1991). Conversely, other studies of sheep have identified different types of teat lesions such as chapping, or teat stenosis as a risk factor for udder infection after experimental exposure to *M. haemolytica*. Teat damage may predispose to bacteria adhesion, invasion and compromise the defensive role of the teat for udder infection (Mavrogianni *et al.*, 2007). However, an observational study in dairy cows demonstrated lower gland SCC with mild to moderate effect of teat end hyperkeratosis (Breen *et al.*, 2009). In our study, the observation at that visit of a teat lesion of any type positioned near the teat orifice was also associated with a decrease in HSCC although there was confounding between this and the variable interacting ewe age and body condition, so it was not included in the multivariable model. However, regard to different management regimes must be made when drawing inference from teat lesions of dairy animals to those of suckler ewes as there is likely to be a difference in aetiology of teat lesions in ewes rearing lambs and mechanically milked dairy ruminants, thus teat lesions may be of a different type and involve different bacteria.

3.5.3 Udder conformation

A greater udder drop was associated with an increase in HSCC, although this did not affect lamb growth. It may be that pendulous udders are more exposed to environmental contamination, thus increasing challenge with minor or major pathogens and an associated increase in SCC. Casu *et al.* (2010) demonstrated that dairy ewes with greater cistern height, decreased degree of udder suspension and

decreased udder drop had higher SCC (determined as at least 2 daily recordings of SCC > 1,000 x 10^3cells/ml over lactation). In dairy cattle there is strong evidence that poor udder conformation is associated with raised somatic cell count and an increased incidence of clinical mastitis (reviewed by Seykora and McDaniel 1985). The current study used a combination of a scoring system and measurement (in centimetres) to evaluate and record udder and teat conformation. Similar approaches have previously been employed for udder conformation assessment of dairy ewes (de la Fuente *et al.*, 1996; Casu *et al.*, 2006 and 2010) and the chart and score system developed by Casu *et al.* (2006) was adapted and used in the current study. Although scoring may be subject to observer bias, Casu *et al.* (2006) demonstrated that this system had fairly high levels of repeatability across lactation in dairy ewes. In the same study, the author found that udder suspension was highly correlated with udder drop (r = 0.82) (Casu *et al.*, 2006), to which our figure (r = 0.80) is comparable. In dairy sheep, linear appraisal of udder traits has been developed (Casu *et al.*, 2006; de la Fuente *et al.*, 1996; Marie-Etancelin *et al.*, 2006). Casu *et al.* (2010) studied a flock of 900 pedigree ewes with historical data and known family relationships and detected a genetic correlation between udder conformation and mastitis and SCC with a heritability of 0.4. Currently, some European dairy sheep breeds include udder traits in their breeding programs, mainly with the aim of improving machine milking ability (Casu *et al.*, 2006; Casu *et al.*, 2010; Marie-Etancelin *et al.*, 2006).

Total teat cross-sectional area was positively associated with HSCC. The minimum and maximum area was 7.5cm^2 and 15cm^2 respectively and for every 1cm^2 increase the $\log_{10}$ HSCC increased by 0.3 log, thus the maximum cross-sectional teat area observed was associated with a HSCC that was around 200 cells/ml higher than the smallest teat. One possible explanation it that a bigger teat cistern may facilitate a

greater volume of residual milk in the teat in which minor or major pathogens may multiply. Another explanation is that bigger teats may have larger teat sphincters with larger orifices which may increase the risk or pathogen entry. However, the diameter of teat sphincters is difficult to measure and was not attempted in this study.

3.5.4 Ewe age and body condition

A higher HSCC was associated with 9 year old ewes that were in BCS of 2.5 or less, and in 6 year old ewes of BCS 1.5 or less, compared with 2 year old ewes that were in BCS of more than 3. It is probable that older ewes have been exposed to more pathogens over the course of multiple lactations, and that a lower BCS was associated with chronic disease or metabolic stress, predisposing to susceptibility to infection. Lafi *et al.* (2006) showed that multiparous dairy ewes had a significantly higher mean ln SCC than primiparous dairy ewes and Watkins *et al.* (1991) showed that prevalence of suckler ewes with subclinical mastitis increased with age of ewe.

Weight of lambs reared by ewes in BCS of 3 or more before lambing was significantly higher than those that were reared by those in BCS of 2 or less before lambing. However, ewe body condition did not change much with time from the first observation pre-lambing until the last observation during the study period. Although body condition scoring is subjective, repeated measures were made blinded by the same observer and observations within ewes showed a high level of consistency. Body condition score and age of ewe were moderately but significantly correlated ($r = 0.62$), thus the association between ewe BCS and lamb weight independent of ewe age is difficult to assess. Al-Sabbagh *et al.* (1995) demonstrated a lower total weaning weight of lambs reared by 7 year old ewes compared with 4 year old ewes, despite the total birth weight being higher in ewes of 7 years. In our study BCS was included in the model of lamb weight but age was not. Body condition score was

highly correlated with each extra day a ewe was fed concentrate feed before lambing compared to the earliest ewe to lamb. The effect of ewe nutrition in late pregnancy with heavier lamb birth weight is documented in the literature (Gardner *et al.*, 2007; Khalaf *et al.*, 1979) and, in concordance with other studies (Greenwood *et al.*, 1998; Green *et al.*, 1998), we also demonstrated that birth weight was a significant factor for greater lamb growth. It was interesting to note that teat position was co-linear with BCS and ewe age and was also not included in the model.

3.5.5 Other significant variables in the lamb weight model

Lambs in which diarrhoea was observed weighed on average 1.15kg less at that observation than those in which no signs of diarrhoea were recorded at a visit. Although the cause of diarrhoea was unknown, the peak incidence was nine weeks after lambing and, when incidence was stratified by study flock and observation, it became evident there was a particular problem within the group of 8 year old mules rearing single lambs, with a prevalence of 72.2% of diarrhoea in lambs in this group at the fourth observation, compared with a prevalence of 0% with diarrhoea in the group of 8 year old mules with twins. Green *et al.* (1998) previously demonstrated the longitudinal effects of diarrhoea on lamb weight, where lambs with diarrhoea experienced a reduction of approximately 2kg in weight over a five week period, compared to lambs without diarrhoea.

Lamb weight was not significantly associated with sex of lamb in the multivariable analysis, although it was significant in the univariable analysis and it has frequently been demonstrated to be a significant factor affecting lamb weight in other studies (Green *et al.*, 1998; Keisler *et al.*, 1992).

There was no evidence that the presence of orf-like lesions anywhere on the lamb's muzzle was associated with a lower lamb weight. There was also no evidence of significant correlation between observation of orf-like lesion on a lamb's mouth and with it being subjectively assessed as either palpably or visibly thin, suggesting that the lesions were not affecting lamb growth. In contrast, Lovatt *et al.* (2012) demonstrated that lambs with orf weighed approximately 10% less for up to five weeks of age than lambs without. The current study farm did not vaccinate for orf and no samples were collected for microbiological identification of the causative organism; therefore diagnosis of orf was provisional, made on gross lesion morphology on clinical examination. The authors are mindful that orf lesions may be easily misdiagnosed with dermatitis lesion caused by S*taphylococcus aureus*.

3.5.6 Other significant variables in the somatic cell count model

Temporal trends of SCC over the first 2 months of lactation were observed in this study with a general decrease in SCC over time after lambing and a subsequent gradual rise in the second half of the observation period. This is a similar pattern to that observed in the study previously conducted on a different farm in the previous year and these temporal trends of SCC have been discussed previously (Chapter 2)

3.5.7 Hygiene variables

Udder contamination was assessed at each observation and was not associated with a significant change in either HSCC or lamb weight at that observation or at the subsequent observation. A longitudinal study in dairy cows did find an association between udder contamination and SCC (Breen *et al.*, 2009). However, udder contamination may be very transient in nature and each ewe was only examined on average every two weeks so there is much potential variability of such a subjective assessment. Neither cleanliness of bedding nor water availability at lambing were

associated with a change in lamb weight or SCC. However both of these variables are difficult to assess and accurately record on a commercial farm. Water availability to each ewe in the periparturient period was observed when ewes were in individual lambing pens and at this time visual cleanliness of bedding within the pen was also recorded but because ewes and lambs were subsequently turned to outdoor pasture, assessment of environmental hygiene and water availability throughout the rest of the study was impractical.

3.5.8 Importance of study

This is the first study to demonstrate the association between udder conformation and teat damage of suckler ewes with SCC and lamb weight and has thus contributed to the knowledge of the relationships between lamb weight and SCC. The findings suggest that the risk of IMI may be reduced and lamb production may therefore be optimised by management choices employed by the sheep farmer. For example ewe replacement and culling choices should select for ewes and progeny with good udder conformation to remain in the flock whilst avoiding retaining ewes with low udder drop and large teats. The avoidance of retaining old ewes in the flock may be beneficial. Ewes must be fed appropriately during gestation and lactation to ensure that milk production meets lambs' demands and this may be monitored by the farmer by observing ewe BCS through lactation and tipping the ewes during lactation to observe for teat lesions, the presence of which may also indicate that there may be a infection process occurring either in the udder, or systemically, affecting milk production. Methods in which teat lesions may be prevented and how udder conformation affects subclinical infection of the udder need further investigation.

3.6 CONCLUSIONS

This study demonstrated negative associations between somatic cell count and lamb weight, between traumatic teat lesions and lamb weight and between udder conformation and SCC. These findings not only suggests that subclinical intramammary infection has an adverse effect on lamb weight but that other aspects of udder health also have an important effect on production. Lower lamb weights were observed in lambs reared by ewes with teat damage and those with somatic cell counts greater than 400,000 cells ml. Lambs reared by ewes in BCS 2.5 or less weighed less on average than those in BCS 3 or more. Elevated somatic cell counts were present in older and thinner ewes, and in those with a greater drop of the suspended udder and larger teats, indicating that these ewes have higher levels of subclinical udder infection.

Other factors associated with lamb weight were age of lamb, lamb birth weight, litter size, age of ewe, number of days a ewe received supplementary concentrate feed before lambing and whether or not the lamb had diarrhoea, whilst SCC pattern was described by days in lactation and average weight of nursing litter.

This study successfully demonstrated the importance of overall udder health and identified some potential practical approaches that farmers can make which should be tested in intervention trials. These include selecting against ewes with poor udder conformation and monitoring ewe body condition to inform appropriate feeding of ewes through late gestation and lactation. These approaches may form part of a beneficial farm management strategy in optimising flock udder health.

4. CHAPTER 4: A STUDY TO INVESTIGATE THE EFFECT OF DRY COW THERAPY ON INTRAMAMMARY INFECTION IN THE FIRST 2 MONTHS OF THE SUBSEQUENT LACTATION IN SUCKLER EWES.

4.1 ABSTRACT

Research in dairy cows has demonstrated that the use of dry cow therapy (DCT) reduces intramammary infection (IMI) and improves milk yield in the subsequent lactation. A cohort study was conducted on one farm to assess the effect of the use of DCT on lamb growth and somatic cell count. Weights of 194 lambs reared by 108 suckler ewes, 56 of which had received DCT, were recorded at lambing and at fortnightly intervals until lambs were eight to ten weeks old. Milk samples were collected for udder half somatic cell counting (HSCC) from 33 ewes (17 of which had received DCT when weaned), at lambing, at one month and two months into lactation.

A multilevel model was constructed with weight of lamb as the outcome variable and three random effects for ewe, lamb and repeated measures of weight. There was no significant difference in weight of lambs reared by ewes that had received DCT, compared to lambs reared by ewes that had not received DCT. The effect of DCT on HSCC was assessed using ANOVA. There was no significant difference between $\log_{10}$ HSCC of treated and untreated groups at during lactation. However, a significant difference between $\log_{10}$ HSCC across months of lactation (P>F=0.034) was observed in treated ewes but not in untreated ewes. This difference was explained by a significant decrease in HSCC between weaning and lambing ($p = 0.002$) and between one and two months in lactation ($p = 0.045$). Udder half somatic

cell counts were significantly lower between 1ambing and 1 month in lactation (p = 0.021) in untreated ewes.

4.2 INTRODUCTION

As previously demonstrated (Chapter 3), ewes with elevated milk SCC rear lighter lambs. The aim of this study was to assess whether ewes which received DCT had lower HSCC and reared heavier lambs over the first 2 months of the subsequent lactation.

4.3 MATERIALS AND METHODS

4.3.1 Study farm and ewe selection

A farm in Shropshire, England that had been used as a study farm for the cohort study investigating the association between lamb weight and somatic cell count (Chapter 3) was convenience selected as the study farm.

The number of ewes required for the study was calculated using assumptions made from observations of untreated ewes and lambs from the same farm in the previous year. The assumptions were that each ewe reared an average of 1.5 lambs, that the mean lamb weight at 8 weeks of age was 17.0kg (95% CI: 8.0 , 26.0) and lambs weighed on average 1.70kg less (95% CI: -2.36, -1.04) when the ewe mean SCC was >400,000 cells/ml. Ninety-one lambs reared by ewes that received DCT and 91 lambs reared by ewes that did not receive DCT were required to detect a 1.70kg difference in lamb weight at 8 weeks of age, with 95% confidence and 80% power. Thus 61 ewes were required to receive DCT, and 61 ewes to remain untreated.

A total of 152 ewes were enrolled into the study at weaning in August 2010. According to age at lambing 2011, the study group comprised 70 3 year old North Country mules and 82 6 year old North Country mules.

4.3.2 Collection of ewe and lamb data

A sub-selection of 43 ewes comprising 20 3 year old ewes and 23 6 year old ewes were randomly selected for milk sampling at time of enrolment and the following time points: 1-4 days of lambing, at approximately 4 weeks and approximately 8 weeks into lactation. On the same occasions, prior to milk sample collection and whilst the ewe was in pelvic recumbency, observations of new teat lesions were recorded. At 1 month in lactation, udder measurements and scores were taken with the ewe standing and in pelvic recumbency according to methods based on Casu *et al.* (2006) and as described in Chapter 3. Observations of udder measurement, scores and teat abnormalities were made by the same observer as Chapter 3.

Lamb weights from lambs reared by untreated and treated ewes were measured at lambing and then at 2-week intervals until lambs were about 10 weeks of age.

4.3.3 Collection of milk samples

Milk samples for udder half somatic cell counting were aseptically collected according to the methodology described in Chapter 2.

4.3.4 Administration of intramammary antibiotic

Seventy-two ewes were randomly selected for treatment (37 3 year old ewes and 35 6 year old ewes) at weaning. In ewes that were selected to be treated, gross faecal or soil contamination of each udder half was removed with a coarse paper towel which was discarded. The teat end was then cleaned twice with 70% ethanol. The entire contents of a 5ml intramammary antibiotic treatment tube containing 100mg

framycetin sulphate, 100mg penthemate hydrochloride and 300mg procaine penicillin (Ubro Red, Boehringer Ingelheim) was aseptically administered into the udder half. The process was repeated for the other udder half, using a fresh tube.

4.3.5 Data storage and analysis

A database was constructed in Microsoft Access 2007 into which data of observation date, ewe ID, whether a ewe received treatment, body condition score, HSCC (where collected), udder conformation scores, abnormalities of the udder, teat and milk, lambing date, lamb ID, sex, litter size, lamb weight and the observation of diarrhoea or orf on a lamb were stored. Raw HSCC values were received from the lab, corrected to 1 x 10^3 cells/ml with one decimal place. Raw HSCC values were log transformed to normalise the data. Log_{10} mean somatic cell count for each ewe at each observation was categorised into quintiles.

Data were retained for analysis from lambs that were reared by ewes for which data from at least one lamb with more than two observations of lamb weight between lambing and 8 weeks of age were available. Lambs with two or fewer observations were excluded from the analysis.

Descriptive analysis was performed in Stata 10 (StatCorp LP, Texas) using methodology as previously described in chapter 4. ANOVA of HSCC was conducted in Stata 10. Teat abnormalities that were bites, tears or chapping were classified as traumatic teat lesions whilst those that were pustules, warts or orf-like lesions were classed as non-traumatic according to the method developed by Cooper (2011, Master's thesis).

Multilevel modelling was performed in MLwIN 2.11 (Rasbash *et al.*, 2009). A three-level linear multivariable regression model was constructed to analyse the data with

lamb weight (kg) as the continuous outcome variable with ewe, lamb and observation as levels 3, 2 and 1 random effects as described in Chapter 3. The model took the general structure:

$$y_{ijk}=\beta_0+ \beta x_{ijk} + \beta x_{jk} + \beta x_k + v_{0k} + u_{0jk} + e_{0ijk}$$

where y_{ijk}~N(XB, Ω)

and where y*ijk* was the continuous outcome variable of lamb weight (kg) and β was a series of fixed effects that varied at levels *ijk* (observation), *jk* (lamb) and *k* (ewe), with variance estimates *v*, *u* and *e*. The independent variables were tested in the model using a manual forward stepwise selection process. Significance was set at 0.05.

4.4 RESULTS

At weaning, there were 152 ewes enrolled in the study, of which 72 (47%) were treated with Ubro Red and 43 (28%) were milk sampled for HSCC. Between weaning and lambing 31 (20%) of these ewes were lost from the study due to conception failure or death. At lambing, one ewe developed clinical mastitis and was excluded from the study. Out of 236 lambs born to 121 study ewes, 42 (18%) were lost from the study due to death or adoption on to a non-study ewe.

After the exclusion of data from ewes and lambs with incomplete or truncated records, the dataset for analysis consisted of complete data from 108 ewes that lambed over a period of 46 days and reared 194 lambs. There were 948 observations of lamb weight from lambing to two months of age. The oldest lamb at the last observation was 70 days old.

4.4.1 Descriptive analysis of ewe data

Out of 108 ewes in the study, 47 (44%) were 3 years old and 61 (56%) were 6 years old, with 20% of ewes rearing single lambs and the remainder rearing twins (Table 4.1).

Table 4.1 Number of lambs reared in each ewe age group

Ewe age (years)	singles	twins	Total
3	7	40	47
6	15	46	61
Total	22	86	108

The majority of ewes were in BCS of 2.5 across both age groups at lambing and remained constant over the first two months in lactation, with the exception of 6 year old ewes rearing twins most of whom were in BCS 3 at lambing but lost one unit of BCS during the first two months of lactation.

Of the 108 ewes in the data set, 56 had received intramammary antibiotic treatment at weaning whilst 52 had not received treatment. The number of ewes sampled in each group was approximately equal (Table 4.2.)

Table 4.2. The number of ewes treated and milk sampled for somatic cell counts in each age group

	3 year olds			6 year olds			Total
SCC	Untreated	Treated	subtotal	Untreated	Treated	subtotal	
Unsampled	12	19	31	24	20	44	75
Sampled	8	8	16	8	9	17	33
subtotal	20	27	47	32	29	61	108

4.4.2 Udder conformation and damage

At weaning the prevalence of palpable or visible udder abnormalities in ewes was low. Of all 152 ewes enrolled in the study, 4% of ewes had udder abnormalities at weaning, recorded as a palpable intramammary mass or mastitis or blood or clots in the milk/transition fluid, and 14% had a scar on at least one teat. Of 108 ewes

retained in the data set for analysis, the prevalence of ewes with a recorded abnormality of the mammary gland or scarred teats at weaning was very similar, 4% (n=6) and 13% (n=12) respectively, suggesting that ewes were not more likely to drop out of the study because of teat or udder damage.

Out of 251 observations of teats for teat lesions at lambing, one month and two months in lactation on 66 teats from 33 ewes, there were 58 observations of at least one teat lesion that was traumatic or non traumatic (23.11%), 46 observations of at least one traumatic teat lesion (18.33%) and 23 observations of at least one non-traumatic teat lesions (9.16%). The majority of teats (n= 40, 61.90%) and ewes (n=25, 75.75%) had either a traumatic or a traumatic teat lesion on at least one occasion over the observation period (Table 4.3).

Table 4.3 Summary statistics and observations for categorical variables

Categorical variables	**n observations**	**N**	**% of observations**
Ewe age (at lambing)			
3yr	47	108	43.52
6yr	61	108	56.48
Litter size			
one lamb	22	108	20.37
two lambs	86	108	79.63
Teat placement scores			
1	1	30	3.33
2	1	30	3.33
3	3	30	10.00
4	7	30	23.33
5	7	30	23.33
6	7	30	23.33
7	4	30	13.33
8	0	30	0.00
9	0	30	0.00
Degree of udder separation (score)			
1 (minimum separation)	16	30	53.33
2	10	30	33.33
3	3	30	10.00
4	1	30	3.33

5-9	0	30	0.00
Weaning BCS			
1	2	107	1.87
1.5	7	107	6.54
2	29	107	27.10
2.5	46	107	42.99
3	15	107	14.02
3.5	7	107	6.54
4	1	107	0.93
BCS before lambing			
1.5	1	107	0.93
2	5	107	4.67
2.5	20	107	18.69
3	39	107	36.45
3.5	37	107	34.58
4	5	107	4.67
BCS before lambing (4 categories)			
2 or less	6	107	5.61
2.5	20	107	18.69
3	39	107	36.45
3.5 or more	42	107	39.25
BCS at monthly observation			
1	1	97	1.03
1.5	2	97	2.06
2	28	97	28.87
2.5	38	97	39.18
3	25	97	25.77
3.5	3	97	3.09
BCS at monthly observation			
1.5 or less	3	97	3.09
2	28	97	28.87
2.5	38	97	39.18
3	25	97	25.77
3.5	3	97	3.09
4 or more	0	97	0.00
Ewe had either a traumatic or a non-traumatic teat lesion on at least one teat at any point through entire study period	25	33	75.75
Ewe had a traumatic teat lesion on at least one teat at any point through entire study period	17	33	51.52
Ewe had a non-traumatic teat lesion on at least one teat at any point through entire study period	15	33	43.75
Teat had either a traumatic or a non-traumatic teat lesion at any point through entire study period	40	66	60.61
Teat had a traumatic teat lesion at any point during the entire study period	30	66	45.45
Teat had a non-traumatic teat lesion at any point during the entire study period	21	66	31.83

Teat had traumatic or non traumatic lesion at visit	58	251	23.11
Teat had traumatic lesion (bites, tears, chapping) at visit	46	251	18.33
Teat had non-traumatic lesion (orf, warts, spots) at visit	23	251	9.16
Observation of a lamb with diarrhoea at visit	94	948	9.92
Lamb had diarrhoea at at least one visit	50	194	25.77
Lamb had orf-like lesion at visit	0	948	0.00

Measurements of drop and width of udder of 108 ewes retained for data analysis showed a wide range of values. The minimum, mean and maximum lengths and widths (cm) of left and right teats were almost identical. The minimum, mean and maximum HSCC (x 10^3 cells/ml) of left and right udder halves were also very similar. The last ewe to lamb received supplementary concentrated feed before lambing for 46 days longer than the first ewe to lamb (Table 4.4).

Table 4.4 Summary statistics and observations for continuous variables

Continuous variables	**Min**	**Max**	**Mean**	**Std. Dev.**	**n obs**
Lamb age (days)	0	70	29	20.11	948
Birth weight (kg)	1.9	9.6	5.09	1.11	194
Biweekly lamb weight (kg)	1.9	35.5	12.55	6.15	948
Number of days ewe fed concentrates before lambing *(first ewe to lamb as ref)*	0	46	15.83	6.80	108
Log SCC left udder-half from lambing to 2 months	4.74	6.41	5.45	0.37	80
Log SCC right udder-half from lambing to 2 months	4.81	6.46	5.51	0.42	79
Log SCC all udder-halves from lambing to 2 months	4.74	6.46	5.48	0.39	159
SCC left udder half (x 10^3 cells/ml) from lambing to 2 months	55	2554	411.04	430.57	80
SCC right udder half (x 10^3 cells/ml) from lambing to 2 months	65	2912	529.99	624.34	79
SCC all udder halves (x 10^3 cells/ml) from lambing to 2 months	55	2912	470.14	533.84	159
Drop of udder (cm)	12.0	21.0	16.77	2.08	30
Width at base of udder (cm)	15.4	23.0	19.41	1.90	30
Left teat length (cm)	2.3	3.8	2.99	0.32	30
Right teat length (cm)	2.3	3.9	3.12	0.40	30
Left teat width (cm)	1.1	2.8	1.61	0.33	30
Right teat width (cm)	1.2	2.9	1.75	0.35	30
Sum cross sectional area of both teats (cm^2)	6.25	17.1	10.34	2.61	30

4.4.3 Somatic cell count

There were 159 observations of SCC from 65 udder halves from 33 ewes at two to three days after lambing and at approximately one month and two months into the study. Some of these ewes had already started to dry off at weaning, thus there were observations from fewer udder halves (n=59) from fewer ewes (n=32) at weaning.

Over all observations of SCC in lactation, the arithmetic mean was 309 x 10^3 cells/ml, the mean $\log_{10}$ SCC was 5.48 and the geometric mean was 303 x 10^3 cells/ml. The mean fifth quintile value for mean ewe somatic cell count at each observation was ten times that of the first quintile mean value (Table 4.5)

Table 4.5 Quintiles of mean ewe somatic cell counts

Quintile	Min ewe SCC		Mean ewe SCC		Max ewe SCC	
	cells/ml	$\log_{10}$ cells/ml	Arithmetic mean (cells/ml)	$\log_{10}$ cells/ml	cells/ml	$\log_{10}$ cells/ml
1st	62500	4.80	103500	5.02	134000	5.13
2nd	134000	5.13	192400	5.28	249000	5.40
3rd	249000	5.40	282800	5.45	326000	5.51
4th	337000	5.53	458700	5.66	644000	5.81
5th	656000	5.82	1361300	6.13	2912000	6.46

NB Raw SCC results submitted by lab as 3 or 4 significant figures (s.f.)

Milk somatic cell counts were highest at weaning and then decreased over the subsequent stages in lactation: at lambing, one and two months in lactation although this difference was not significant in the combined treatment groups (Figure 4.1 and Table 4.6).

Figure 4.1 Log_{10} HSCC by stage in lactation

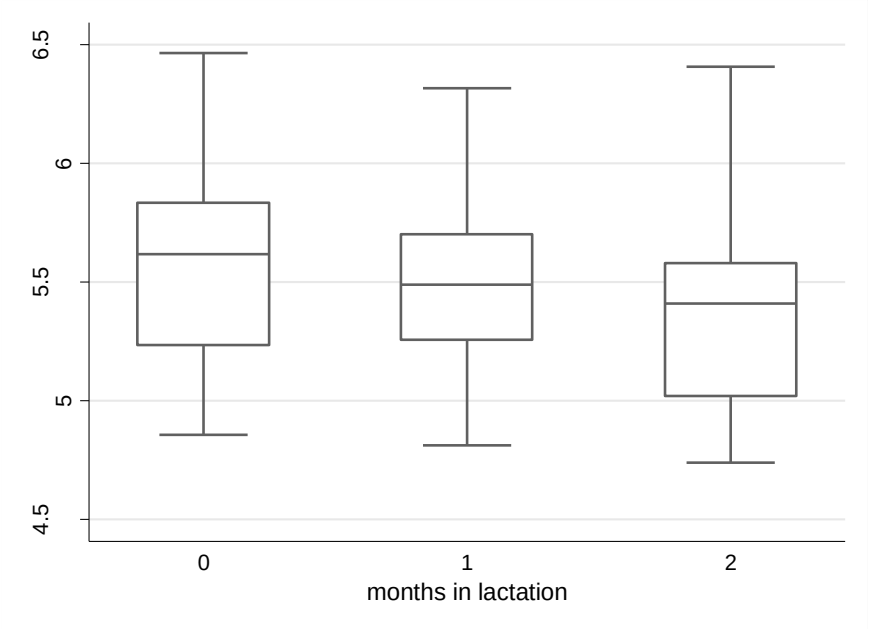


Table 4.6 Mean log_{10} HSCC by observation event

Observation event	Mean	95%CI		Median	Coefficient of Variance	N udder halves
		lower	upper			
Weaning	5.89	4.93	6.85	5.80	0.08	59
Lambing	5.60	4.84	6.36	5.62	0.07	41
1 month in lactation	5.51	4.78	6.23	5.49	0.07	61
2 month in lactation	5.37	4.58	6.16	5.41	0.07	57

There were a total of 67 observations of untreated udder halves and 81 from treated udder halves, from weaning, at lambing and then at approximately one and two months in lactation (Table 4.7).

Table 4.7 Log_{10} HSCC by months in lactation in untreated and treated ewes

Observation	Untreated					Treated				
	mean	95% CI		CV	N	mean	95% CI		CV	N
event		lower	upper				lower	upper		
Weaning	5.86	4.76	6.96	0.10	27	5.91	5.07	6.75	0.07	32
Lambing	5.85	4.66	7.05	0.10	16	6.02	5.20	6.84	0.07	22
1 month in lactation	5.98	4.97	6.98	0.09	27	5.82	5.13	6.51	0.06	28
2 months in lactation	5.86	4.72	7.00	0.10	24	5.92	5.06	6.77	0.07	31
Total observations					67					81

4.4.4 Difference in somatic cell count between groups by stage of lactation

At the point of administration of treatment at weaning there was no significant difference between $\log_{10}$ HSCC of treated and untreated groups (Table 4.8). In the subsequent lactation there was no significant difference between $\log_{10}$ HSCC of treated and untreated groups at lambing, or at one month or two months in lactation although the variance between treated and untreated groups was not equal in the first month of lactation (Bartlett's test for equal variances, $P> \chi^2 = 0.03$) (and Table 4.9). However in treated ewes, there was a significant difference between $\log_{10}$ HSCC across months ($P = 0.034$) although no difference was seen between months of lactation in untreated ewes (Table 4.10). This difference across months for treated ewes was explained by a significant decrease in $\log_{10}$ HSCC between weaning and lambing ($p = 0.002$) (Table 4.11, Table 4.2) and between one and two months in lactation ($p = 0.045$) (Table 4.13) although there was no significant difference in $\log_{10}$ HSCC between lambing and 1 months in lactation. For untreated ewes $\log_{10}$ HSCC was significantly lower between 1ambing and one month in lactation ($p = 0.021$) (Table 4.12).

Table 4.8 T-test for difference in $\log_{10}$ HSCC in untreated and treated ewes at weaning

Two-sample t test with equal variances				95% CI	
Variable	Obs	Mean	Std Error	lower	Upper
untreated	24	5.84	0.11	5.61	6.06
treated	26	5.98	0.09	5.80	6.15
combined	50	5.91	0.07	5.77	6.05
mean(untreated)-mean (treated)		-0.14	0.14	-0.41	0.14
mean(diff) = mean weaning SCC (untreated ewes-treated ewes)				df=48	t= -0.999
Ho:diff=0		Ha: diff>0			Pr(T>t)=0.839

Figure 4.2 Log_{10} HSCC by months in lactation in untreated and treated ewes

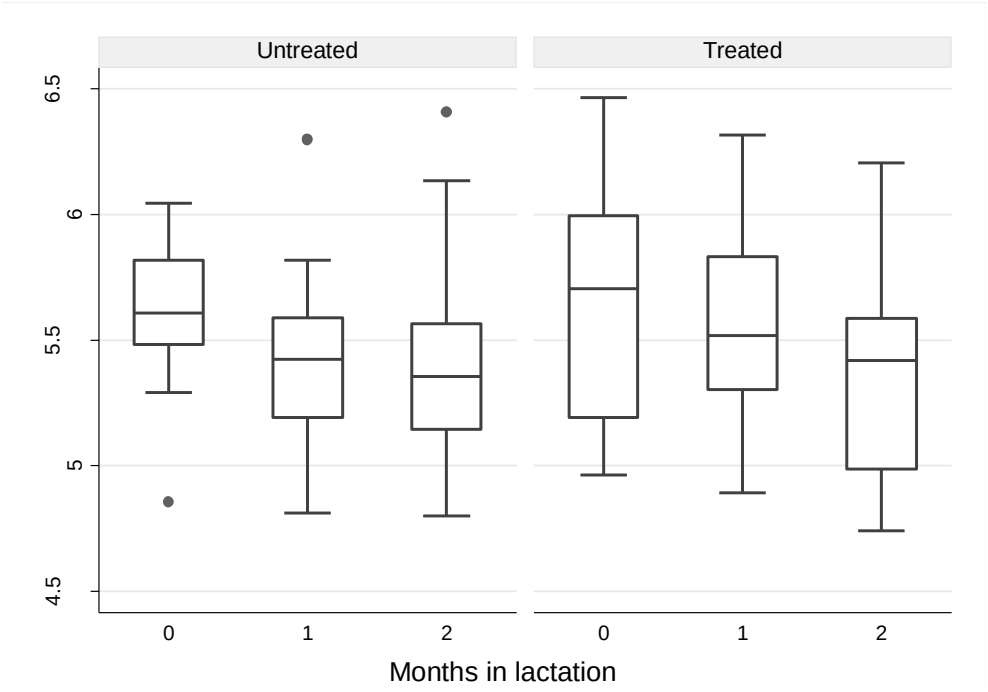


Table 4.9 One way ANOVA for difference in log_{10} HSCC in untreated and treated ewes by month of lactation

Lambing		Number of obs F test statistic (F) Prob >f	40 0.01 0.92
	Sum of squares (SS)	Degrees of freedom (df)	Mean Square(MS)
Between treatment groups	0.00	1	0 .00
Within groups	5.99	39	0.15
Total	5.99	40	0.15
Bartlett's test for equal variances		χ^2 (2)= 4.53	Prob> χ^2 = 0.03
One month in lactation		Number of obs F Prob >f	60 1.25 0.2674
	SS	Df	MS
Between treatment groups	0.17	1	0.17
Within groups	8.06	59	0.14
Total	8.23	60	0.14
Bartlett's test for equal variances		χ^2 (2)= 0.08	Prob> χ^2 = 0.78
Two months in lactation		Number of obs F Prob >f	56 0.54 0.4673
	SS	Df	MS
Between treatment groups	0.09	1	0 .09
Within groups	8.92	55	0.16
Total	9.01	56	0.16
Bartlett's test for equal variances		χ^2 (2)= 0.001	Prob> χ^2 = 0.94

Table 4.10 One way ANOVA for difference in log_{10} HSCC across months in lactation in untreated and treated ewes

Untreated ewes		Number of obs	74
		F	1.48
		Prob >f	0.234
	SS	df	MS
Between months of lactation	0.40	2	0.20
Within months of lactation	9.70	72	0.13
Total	10.09	74	0.14
Bartlett's test for equal variances		χ^2 (2)= 2.80	Prob> χ^2 = 0.25
Treated ewes		Number of obs	83
		F	3.51
		Prob >f	0.034
	SS	df	MS
Between months of lactation	1.15	2	0.58
Within months of lactation	13.28	81	0.16
Total	14.43	83	0.17
Bartlett's test for equal variances		χ^2 (2)= 1.547	Prob> χ^2 = 0.46

Where months in lactation are lambing, 1 month and 2 months in lactation

Table 4.11 Paired t-test of log_{10} HSCC of milk from untreated and treated ewes between weaning and lambing

Untreated ewes						
Variable	Obs	Mean	Std. Err	Std. Dev.	95% CI	
wean SCC	16	5.85	0.15	0.61	5.53	6.18
lambing SCC	16	5.60	0.07	0.29	5.45	5.76
diff	16	0.25	0.16	0.63	-0.09	0.59
mean(diff) = Mean (wean SCC-lambing SCC)				df=15		t=1.588
Ho: mean(diff) = 0				Ha: mean (diff)> 0		Pr(>t)= 0.067

Treated ewes						
Variable	Obs	Mean	Std. Err	Std. Dev.	95% CI	
wean SCC	22	6.02	0.09	0.42	5.83	6.20
lambing SCC	22	5.57	0.10	0.45	5.36	5.77
diff	22	0.45	0.14	0.66	0.16	0.75
mean(diff) = Mean (wean SCC-lambing SCC)				df=21		t=3.228
Ho: mean(diff) = 0				Ha: mean (diff)> 0		**Pr(>t)=0.002**

Table 4.12 Paired t-test of log_{10} HSCC of milk from untreated and treated ewes between lambing and 1 month in lactation

Untreated ewes						
Variable	Obs	Mean	Std. Err	Std. Dev	95% CI	
lambing SCC	12	5.70	0.06	0.20	5.57	5.82
SCC 1 month in lactation	12	5.49	0.06	0.20	5.37	5.62
diff	12	0.20	0.09	0.31	0.01	0.40
mean(diff) = mean (lambing SCC-1 month SCC)				df=11		t = 2.299
Ho: mean(diff)=0				Ha: mean(diff) >0		Pr(T> t) = **0.021**

Treated ewes						
Variable	Obs	Mean	Std. Err	Std. Dev	95% CI	
lambing SCC	19	5.60	0.10	0.43	5.39	5.81
SCC 1 month in lactation	19	5.55	0.09	0.38	5.37	5.73
diff	19	0.05	0.08	0.36	-0.12	0.22
mean(diff) = mean (lambing SCC-1 month SCC)				df=18		t = 0.6040
Ho: mean(diff)=0				Ha: mean(diff) >0		Pr(T> t) = 0.277

Table 4.13 Paired t-test of log_{10} HSCC of milk from untreated and treated ewes between 1 and 2 months in lactation

Untreated ewes						
Variable	Obs	Mean	Std. Err.	Std. Dev.	95% CI	
					lower	upper
one month in lactation	19	5.56	0.08	0.34	5.39	5.73
two months in lactation	19	5.45	0.10	0.44	5.24	5.66
diff	19	0.11	0.13	0.56	-0.16	0.38
mean(diff) = mean (one month SCC-2 month SCC)				df=18		t = 0.8886
Ho: mean(diff)=0				Ha: mean(diff) >0		Pr(T> t) = 0.23

Treated Ewes						
Variable	Obs	Mean	Std. Err.	Std. Dev.	95% CI	
					lower	upper
one month in lactation	22	5.52	0.08	0.37	5.36	5.69
two months in lactation	22	5.32	0.09	0.43	5.13	5.51
diff	22	0.20	0.11	0.52	-0.33	0.43
mean(diff) = mean (one month SCC-2 month SCC)				df=21		t=1.7781
Ho: mean(diff)=0				Ha: mean(diff) >0		Pr(T> t) = **0.045**

4.4.5 Difference in somatic cell counts between groups by age of ewe

Within age groups, there was no difference between log_{10} HSCC of 3 year old ewes receiving treatment and ewes not receiving treatment at weaning over all observations of SCC taken between lambing and two months in lactation (p = 0.28) (on a two sample t-test of equal variances with difference between means of 0.01 (-0.08, 0.26), t = 1.09, 80 df)) or of 6 year old ewes (p = 0.22) (on a two sample t-test of equal variances with a difference between means of -0.11 (-0.30,0.07), t = -0.1, 75 df)).

Table 4.14 Log_{10} HSCC over all observations during lactation in untreated ewes and untreated ewes in each age group.

Age Group	Untreated				Treated				Total obs
	Mean	95% CI lower	95% CI higher	N obs	Mean	95% CI lower	95% CI higher	N obs	
3 yr	5.57	5.45	5.69	39	5.47	5.35	5.60	43	82
6 yr	5.38	5.26	5.50	36	5.50	5.36	5.63	41	77
All ages	5.48	5.39	5.56	75	5.48	5.39	5.57	84	159

4.4.6 Teat lesions

Of 33 ewes which were followed for milk sampling at lambing and at one and two months in lactation, there were 79 observations of new teat abnormalities from 22 ewes. Of these, 53 observations were of traumatic type lesions and 26 were of non-traumatic type lesions. Of the observations of a traumatic type, 31 were of bite lesions, 16 were of chapped skin and 6 were of torn teats. Of the observations of the non traumatic type, 13 observations were of pustules, 11 were of warts and two were of orf-like lesions. The peak prevalence for both traumatic and non-traumatic lesions was at one month in lactation (Figure 4.3).

Treatment was associated with a lower risk of traumatic teat lesions, with 88% untreated udder halves observed for teat lesions having traumatic teat lesions compared to 63% of treated udder halves (p = 0.01). There was no effect of treatment

on the prevalence of non-traumatic teat lesions (Table 4.15) and the prevalence of non-traumatic teat lesions was lower than traumatic teat lesions overall.

Figure 4.3 Numbers of new observations of new traumatic and non-traumatic teat lesions by lactation stage.

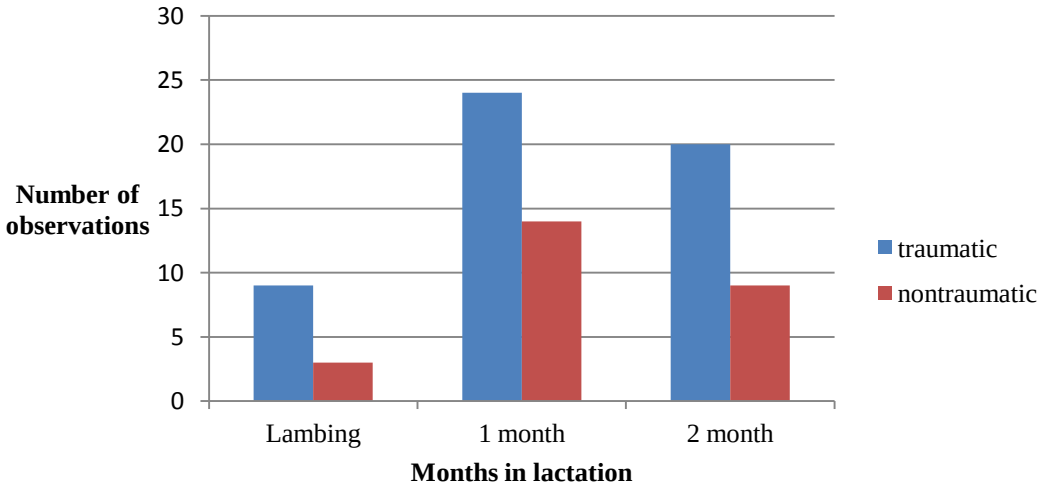


Table 4.15 Two sample test of difference in proportion of teat lesion type in treated and untreated ewes

Traumatic Teat lesions				**N obs**
Two-sample test of proportion		Untreated udder halves		32
		Treated udder halves		27
Variable	Mean	Std. Error	95% CI	
			lower	higher
Untreated	0.88	0.06	0.76	0.99
Treated	0.63	0.09	0.45	0.81
diff	0.25	0.11	0.03	0.46
H_0 diff=Prop (untreated)-Prob (treated)	0.11		2.21	0.027
				z =2.2072
Ho:diff=0	Ha: diff>0			Pr(Z>z)=0.014

Non-traumatic teat lesions				**N obs**
Two-sample test of proportion		Untreated udder halves		32
		Treated Udder halves		27
Variable	Mean	Std. Error	95% CI	
			lower	higher
Untreated	0.31	0.08	0.15	0.47
Treated	0.44	0.10	0.26	0.63
diff	-0.13	0.13	-0.38	0.11
H_0 diff=Prop (untreated)-Prob (treated)	0.13		-1.04	0.296
				z=-1.0441
Ho:diff=0	Ha: diff>0			Pr(Z>z)=0.852

4.4.7 Correlations

Lamb weight was correlated with birth weight (0.65) and ewe BCS (assuming uniformity of BCS between observations). Lamb age was highly correlated with ewe body condition score at visit (0.81) and the observation of diarrhoea (0.71), whilst ewe BCS (at visit) was highly correlated with ewe BCS at weaning (0.92). Left and right $\log_{10}$ HSCC were moderately correlated (0.65).

4.4.8 Descriptive analysis of lamb data

Out of 194 lambs, there were more female lambs (n=106 (55%)) than male lambs (n=87 (45%)); the sex of one lamb was not recorded. During the observation period 50 (26%) lambs had diarrhoea on at least one occasion, with the peak of lambs being seen with diarrhoea at 8 weeks of age (30% of lambs). No lambs had orf-like lesions at any visit (Table 4.3).

4.4.9 Lamb weight

The lowest recorded weight of 1.9 kg was of a lamb that was less than 24 hours old and the highest recorded weight of 35.5kg was from a lamb that was 66 days old, although the oldest lamb to exit the study was 70 days old (Table 4.4). There was a large range of lamb weight (1.9kg to 9.6kg) in the first week after birth (week 0), with a mean of 5.1kg, although lamb age at observation ranged from less than 24 hours to six days. The majority of lambs were eight to nine weeks of age at their last observation, with a mean weight of 20.7 kg, although there was a noteworthy outlier in this week with an observation of one lamb which weighed 35.5 kg; the same lamb had weighed 7.0 kg at 4 days of age. Average lamb weights by week of age (Table 4.16) were linear and exhibited similar variation over weeks 0-8 (Figure 4.4).

There were 432 observations of lamb weight of lambs reared by untreated ewes and 516 observations of lamb weight of lambs reared by treated ewes. There was no

difference in the lamb weight by week of age in lambs reared by treated or untreated ewes (Table 4.17 and Figure 4.5).

Table 4.16 Lamb weight by week of age

Week of age	mean (kg)	95%CI lower	95%CI upper	Median (kg)	Coefficient of Variation	N
0	5.09	2.92	7.26	5.10	0.22	194
1	6.96	4.42	9.49	6.80	0.19	113
2	9.47	4.94	14.00	9.20	0.24	54
3	11.23	6.69	15.76	10.90	0.21	104
4	13.24	9.52	16.95	13.00	0.14	97
5	14.49	8.88	20.09	14.45	0.20	56
6	17.55	12.42	22.67	17.10	0.15	118
7	19.28	13.43	25.12	19.50	0.15	89
8	20.72	14.21	27.24	20.55	0.16	114
9	23.10	10.36	35.84	22.80	0.28	7
10	19.25	16.06	22.44	19.25	0.08	2

Figure 4.4 Weight of lambs by week of age

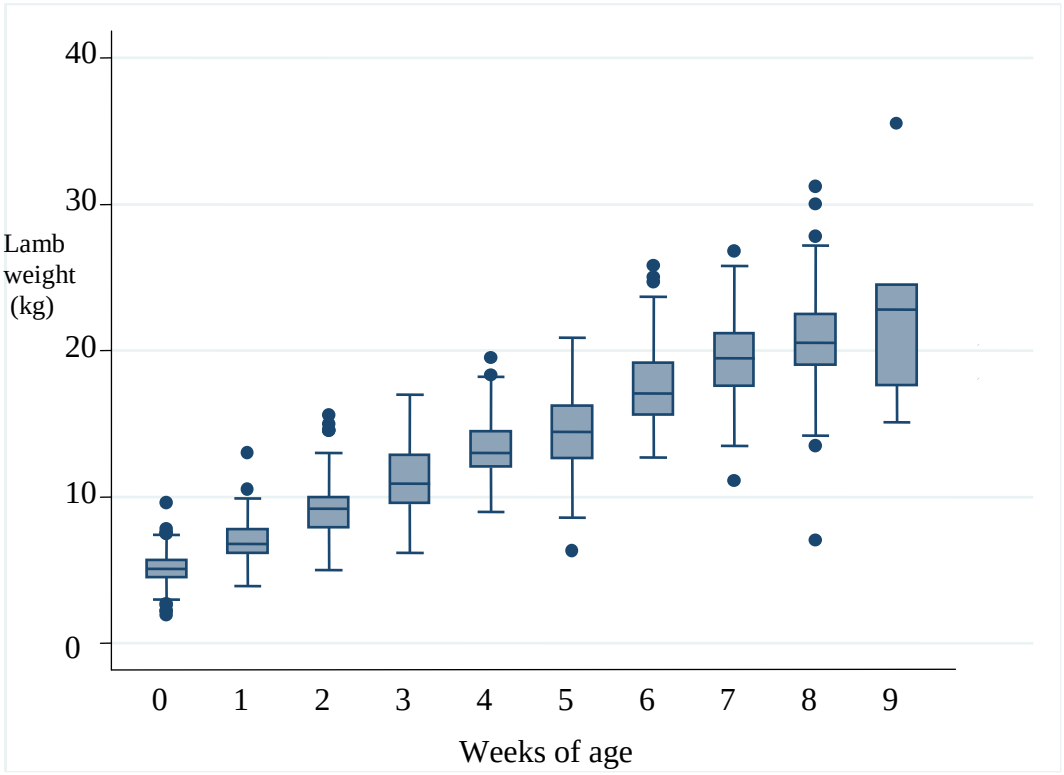
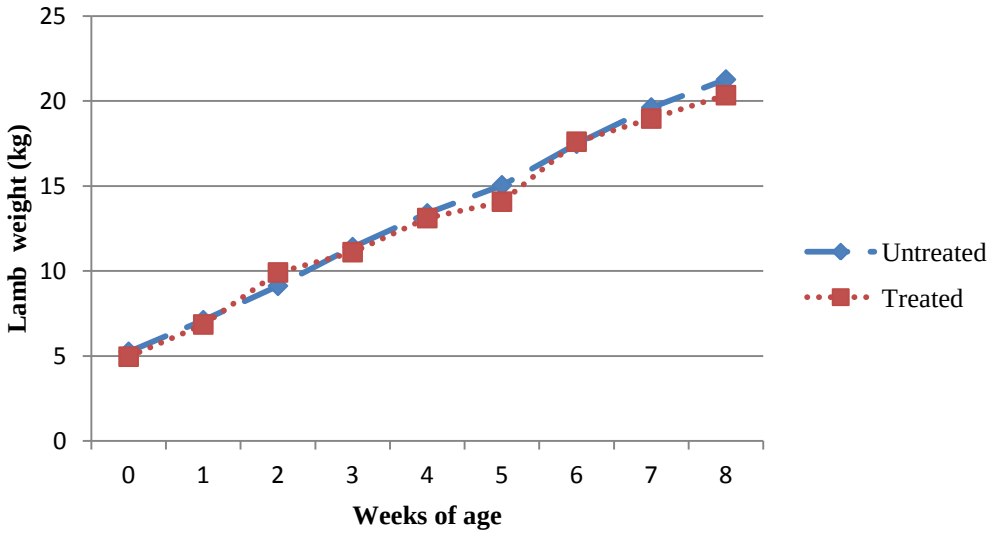


Table 4.17 Lamb weight by weeks of age by treatment group

Weeks of age	Untreated					Treated				
	Mean (kg)	95% CI lower	upper	CV	N obs	Mean (kg)	95% CI lower	upper	CV	N obs
0	5.25	2.94	7.55	0.22	91	4.95	2.94	6.97	0.21	103
1	7.10	4.72	9.49	0.17	49	6.85	4.21	9.49	0.20	64
2	9.12	5.76	12.48	0.19	30	9.91	4.28	15.53	0.29	24
3	11.40	7.03	15.77	0.20	43	11.10	6.42	15.78	0.21	61
4	13.38	9.82	16.95	0.14	46	13.11	9.24	16.97	0.15	51
5	15.04	8.92	21.15	0.21	24	14.07	8.94	19.21	0.19	32
6	17.48	12.25	22.71	0.15	52	17.60	12.52	22.67	0.15	66
7	19.61	13.71	25.52	0.15	43	18.96	13.18	24.74	0.16	46
8	21.26	13.47	29.04	0.19	48	20.33	14.99	25.68	0.13	66
9	21.03	13.49	28.58	0.18	6	35.50	-	-	-	1
10	-	-	-	-	0	19.25	16.06	22.44	0.08	2
Total	12.65	0.41	24.88	0.49	432	12.47	0.54	24.39	0.49	516

Figure 4.5 Mean lamb weight over the first 8 weeks of age of lambs reared by treated and untreated ewes



4.4.10 Difference in lamb weight by ewe age group

Whilst there was no significant difference in mean lamb weights over all observations of lambs reared by untreated 3 year old ewes compared to lambs reared by untreated 6 year old ewes (P = 0.32) lambs reared by 6 year old ewes that had received treatment were significantly heavier than those reared by 3 year old ewes

that had received treatment ((P = 0.0023) (Table 4.18 and Table 4.19). However, there was unequal variance in lamb weights between 3 and 6 year old treated ewes (Bartlett's test, P> χ^2 =0.046); weights of lambs reared by 6 year old ewes showed less conformity to the Normal distribution, particularly for the treated group (Figure A.4.1), thus this comparison between ewe age groups is not robust.

Table 4.18 One way ANOVA for difference in lamb weights in untreated and treated 3 year and 6 year old ewes

Untreated ewes		Number of obs	431
		F	1.00
		Prob >f	0.32
	SS	df	MS
Between 3 and 6 year old groups	39.02	1	39.02
Within ewe age groups	16756.35	430	38.97
Total	16795.38	431	38.97
Bartlett's test for equal variances		χ^2 (1)=1.3062	Prob> χ^2 = 0.253
Treated ewes		Number of obs	515
		F	9.41
		Prob >f	0.0023
	SS	df	MS
Between 3 and 6 year old groups	342.77	1	342.77
Within ewe age groups	18722.80	514	36.43
Total	19065.57	515	37.02
Bartlett's test for equal variances		χ^2 (1)=3.9870	Prob> χ^2 = 0.046

Table 4.19 Mean lamb weight by week of age of lambs reared by 3 and 6 year old ewes in treated and untreated groups

Lamb weight (kg)				
	Untreated		Treated	
Lamb age (weeks)	3 yr	6yr	3 yr	6yr
0	5.07	5.51	4.88	5.19
1	7.10	7.26	6.76	7.32
2	9.59	10.03	8.93	12.38
3	10.77	13.36	10.43	13.24
4	12.76	14.45	13.21	14.11
5	16.65	15.81	14.22	17.98
6	17.24	18.78	16.89	18.17
7	19.67	19.54	18.99	20.20
8	21.33	21.15	19.48	22.03
9		20.20	35.50	19.25
Over all weeks	12.33	12.93	11.75	13.40

Variables significantly associated with lamb weight in the univariable analysis of lamb weight in the hierarchichal model were age, birth weight and sex of lamb, litter size, and the observation of a traumatic teat lesion on the ewe at least once over the study period. Lambs reared by 3 year old treated ewes were lighter than those reared by 6 year old untreated ewes (Table 4.20).

Table 4.20 Univariable analysis in 3–level model of lamb weight

Variable	Univariable Coefficient	95% CI lower	95% CI upper
Response variable is lamb weight (kg)			
Lamb age (days)	0.28	0.28	0.29
Days of concentrate feed before lambing	-0.02	-0.08	0.04
Birth weight (kg)	1.04	0.70	1.38
Sex of lamb			
male	Reference		
female	-0.90	-1.68	-0.11
Litter size			
one lamb	Reference		
two lambs	-1.93	-3.31	-0.55
Age of ewe			
3 yr	Reference		
6 yr	1.17	0.39	1.95
Ewe received DCT			
No	Reference		
Yes	-0.18	-0.97	0.60
Lamb age (weeks)			
0	Reference		
1	2.19	1.84	2.55
2	4.92	4.40	5.44
3	6.58	6.23	6.93
4	8.69	8.33	9.06
5	10.48	9.95	11.02
6	12.64	12.30	12.98
7	14.34	13.94	14.74
8	16.03	15.66	16.40
9	18.25	16.79	19.71
Wean BCS			
1	0.58	-2.98	4.13
1.5	Reference		
2	-0.86	-2.74	1.01
2.5	-0.46	-2.30	1.38
3	-0.28	-2.29	1.74
3.5	0.03	-2.34	2.39
4	-4.60	-9.19	-0.01

Pre-lamb BCS			
1	no observations		
1.5	Reference		
2	2.70	-2.50	7.89
2.5	3.28	-1.71	8.26
3	2.81	-2.15	7.76
3.5	3.89	-1.07	8.86
4	3.06	-2.23	8.35
Ewe visit BCS			
Missing (coded 0)	-1.35	-4.83	2.14
1	-9.98	-22.40	2.45
1.5	Reference		
2	-0.24	-3.82	3.35
2.5	-0.28	-3.84	3.29
3	-3.34	-7.11	0.42
3.5	-3.58	-8.84	1.69
6 year old untreated ewe	Reference		
3 year old treated ewe	-1.15	-1.99	-0.31
Ewe had either a traumatic or a non-traumatic teat lesion at any point through entire study period[$]			
No	Reference		
Yes	-0.492	-1.91	0.92
Ewe had a traumatic teat lesion at any point during the entire study period[$]			
No	Reference		
Yes	-1.431	-2.84	-0.03
Ewe had a non-traumatic teat lesion at any point during the entire study period[$]			
No	Reference		
Yes	-0.492	-1.91	0.92

$658/960 missing observations

However in the multivariable model of lamb weight, there was no significant difference in weight of lambs reared by ewes that had received dry off treatment at weaning, compared to lambs reared by ewes that had not received treatment (Table 4.21).

Table 4.21 Multivariable analysis of lamb weight without and with teat lesions

Variable	Coefficient	95% CI		Coefficient	95% CI	
		lower	upper		lower	upper
Response variable is lamb weight (kg) (n=948)						
Intercept	-1.37	-4.11	1.38	3.28	0.78	5.78
Lamb age (days)	0.29	0.29	0.29	0.29	0.28	0.29
Birth weight (kg)	0.96	0.80	1.13	0.81	0.56	1.06
Days of concentrate feed before lambing	-0.04	-0.07	0.00	3×10^{-3}	-0.06	0.05
Sex of lamb						
Male	Reference			Reference		
Female	-0.52	-0.86	-0.18	-0.12	-0.80	0.55
Litter size						
One lamb	Reference			Reference		
Two lambs	-1.82	-2.49	-1.14	-2.32	-3.62	-1.02
Lamb has diarrhoea						
No						
Yes	-1.15	-1.51	-0.79	-0.88	-1.50	-0.26
Ewe BCS before lambing						
1.5 or less	Reference			Reference		
2	3.78	0.87	6.68	0.91	-0.50	2.31
2.5	2.88	0.12	5.65	-0.74	-1.74	0.27
3	3.02	0.30	5.75	-0.43	-1.34	0.47
3.5	3.63	0.90	6.36	0.00	0.00	0.00
4 or more	4.00	1.09	6.91	0.00	0.00	0.00
Ewe received DCT at weaning						
No	Reference			Reference		
Yes	0.19	-0.29	0.67	-0.17	-0.95	0.62
Ewe had a traumatic teat lesion at any point during the entire study period						
No				Reference		
Yes				-0.99	-1.84	-0.13
	Variance	**95% CI**		**Variance**	**95% CI**	
		lower	**upper**		**lower**	**upper**
Between ewe residual variance	1.00	0.57	1.43	0.41	-0.17	0.98
Between lamb residual variance	0.44	0.18	0.69	0.60	0.02	1.18
Between visit residual variance	1.98	1.78	2.18	2.27	1.85	2.69
-2 x log likelihood=	3532.838 (930 out of 948 cases used)			1103.455 (284 out of 948 cases used)		

Increase in lamb weight was explained by the age of lamb (days) and birth weight; lambs were 0.81kg heavier for every kg higher birth weight. Lambs reared as twins

weighed 2.32 kg less on average than those reared as singles, whilst lambs in which diarrhoea was observed at least once during the study period weighed 0.88 kg less on average. Lambs reared by ewes on which a traumatic teat lesion was observed weighed 0.99 kg less on average than those lambs that were not although only 33 ewes were observed for teat lesions, and thus this information for only 59 lambs existed. There was a very small positive association between the number of days which a ewe received supplementary feed before lambing and lamb weight although this association was of borderline significance at the 95% level. Lamb weight was not significantly different in male and female lambs or in lambs reared by ewes with lower or higher BCS before lambing. Ewe body condition score did not significantly affect the weight of lambs although this variable was highly correlated with days of supplementary feed before lambing. The inclusion of teat lesions in the model explained the remainder of the residual variance between ewes and some of the residual variance between visits. Some outliers existed but were kept in the model (Figure A.4.2).

To assess the association of somatic cell count on lamb weight a multilevel model was constructed to account for age of lamb and other key variables associated with lamb weight, for those lambs reared by ewes for which observations of SCC were made (Table 4.22). This approach was necessary to account for age of lamb (days) when assessing whether ewes with higher somatic cell counts rear lighter lambs, thus more simple methods of assessing levels of SCC with lamb weight such as ANOVA were not possible. Whilst accounting for key explanatory variables of lamb weight; age, birth weight of lamb, the observation of diarrhoea in the lamb, and being reared as a single or twin lamb, a mean ewe SCC greater than 400, 000 cells/ml, was not

significantly associated with lower or higher lamb weight at that observation. However, there few observations of ewes with very high somatic cell counts.

Although observations of a traumatic teat lesions were significantly associated with lower lamb weights it was not included in this model due to the low number of observations for teat lesions (61 observations out of 148 cases used).

Table 4.22 Multilevel model of lamb weight for 60 lambs reared by 33 ewes for which SCC was observed.

Variable	Coefficient	95% CI		Coefficient	95% CI	
		lower	upper		lower	upper
Response variable is lamb weight (kg)						
Intercept	2.64	0.33	4.94	2.54	0.17	4.91
Lamb age (days)	0.29	0.27	0.31	0.29	0.27	0.31
Birth weight (kg)	0.89	0.57	1.20	0.89	0.57	1.21
Litter size						
One lamb	Reference			Reference		
Two lambs	-3.28	-4.65	-1.91	-3.29	-4.66	-1.92
Lamb has diarrhoea						
No	Reference			Reference		
Yes	-1.38	-2.40	-0.36	-1.37	-2.39	-0.35
Somatic cell count						
SCC <400,000	Reference			Reference		
SCC >400,000	0.24	-0.50	0.97	0.23	-0.50	0.97
Ewe received DCT at weaning						
No	n/a			Reference		
Yes	n/a			0.16	-0.72	1.04
	Variance	**95% CI**		**Variance**	**95% CI**	
		lower	**upper**		**lower**	**upper**
Between ewe residual variance	0.59	-0.35	1.53	0.57	1.51	-0.36
Between lamb residual variance	0.49	-0.56	1.54	0.49	1.54	-0.56
Between visit residual variance	3.36	2.37	4.35	3.36	4.35	2.38
-2 x log likelihood =	632.452 (all 148 cases used)			632.327 (all 148 cases used)		

4.5 DISCUSSION

4.5.1 The effect of dry cow therapy on somatic cell count

A significant reduction in HSCC between weaning and lambing and between one and two months in lactation was observed for ewes that received DCT but not for ewes that did not receive this treatment, although there was no significant difference in HSCC between treatment groups. The significant reduction in milk HSCC of treated ewes at lambing compared to weaning suggests that the treatment was effective in the removal of bacteria in the dry period. Linage *et al.* (2008) also found a significant difference between dry-off SCC and SCC at subsequent lambing following administration with the same product in dairy sheep. It is difficult to say whether this would have been detected as a persistent effect and that a difference between HSCC at lambing and one month in lactation would have been significant in a larger trial. Since HSCC between one and two months in lactation was significantly lower than in one month of lactation, this may suggest that the effect of DCT may be persistent. However, an alternative explanation is that treatment had a significant effect at lambing only and that bacteria entered the udder soon after lambing and HSCC rose again to its former level. If that was the case, the effect of DCT in reducing the levels of somatic cell count was short term and the subsequent significant decrease in HSCC between one and two months in lactation was due to some other unexplained variable. A lack of long term effect of antibiotic dry off treatment throughout lactation in this study is in agreement with a study by Chaffer *et al.* (2003) on dairy sheep.

Somatic cell counts were not significantly different between untreated and treated ewes over all observations or at weaning, lambing, one month and two months of lactation but it should be noted that only 16 untreated ewes and 17 treated ewes were

milk sampled and such a small sample size may provide insufficient power in analysis to detect a difference over four observations. The study farm had a history of a low incidence of clinical mastitis and only one ewe that had been recruited into the study at weaning developed clinical mastitis during the study period.

The findings from this study suggest that antibiotic therapy at weaning is highly unlikely to be of benefit in the reduction of levels of subclinical disease on farms with a low incidence of clinical mastitis. This further highlights the importance of maintaining udder health through other management strategies which include appropriate feeding of ewes through gestation and early lactation, vigilance in observation for udder problems (for example by tipping the ewe to observe for teat lesion in early to mid lactation) and not retaining ewes with poor udder conformation or very old ewes for tupping.

4.5.2 The effect of dry cow therapy on lamb weight

Dry cow therapy had no significant effect on lamb weight on this farm. This is not surprising given that there was no difference in HSCC of milk from treated and untreated ewes. However, antibiotic therapy during the dry period has previously been demonstrated as being beneficial for production both in terms of improving lamb growth (Croft *et al.*, 2000) by reducing levels of clinical mastitis in meat flocks and by improving milk yield in dairy flocks by reducing the incidence of clinical mastitis and reducing levels of subclinical infection on farms.

4.5.3 Other variables explaining lamb weight

Lamb weight could be partially explained, as previously demonstrated (Chapter 3), by lamb age, birth weight and litter size. Ewe body condition score did not significantly affect the weight of lamb although the number of days of extra lamb feed was significant; as previously demonstrated these two variables were highly

correlated. The majority of ewes could be considered to have been in fairly good body condition before lambing. Sex of lamb did not significantly influence lamb weight and as previously discussed in Chapter 3 this is in contrast to other studies. Lambs which had diarrhoea weighed less at that observation than lambs that were not observed as having diarrhoea which is accordance with the findings in Chapter 3. Also in agreement with the findings in Chapter 3, the cohort study on the same farm in the previous year, teat lesions were significant associated with lamb weight; lambs reared by ewes which were observed to have a traumatic teat lesion over the course of the study weighed less on average than those reared by ewes without a teat lesion..

An elevated mean ewe SCC of >400,000 cells ml was not significantly associated with lower lamb weights, in contrast to the findings from Chapter 3, nor were we able to demonstrate a ewe SCC value above which there was a significant association with lower lamb weight,. However, the low number of observations of SCC from ewes throughout the study would have dramatically reduced the power to detect such a difference. Furthermore, there were fewer observations of high SCC during the intervention study when compared to the previous year's study on the same farm, albeit different study flocks.

There are some limitations of the study, which should be considered in extrapolating these results to the sheep industry. Only one farm was used in the study thus robust conclusions on the efficacy of treatment on other farms cannot be drawn. The antibiotic preparation used in the trial was not licensed for use in sheep and was selected for its broad spectrum antibiotic efficacy on IMI and clinical mastitis in cows.

4.6 CONCLUSIONS

Dry cow therapy reduced HSCC at lambing but this effect was short lived and did not reduce the HSCC levels significantly below those of ewes not given DCT. Dry cow therapy was therefore not found to be an appropriate management tool to reduce intramammary infection in a flock with a low level of clinical mastitis.

5. CHAPTER 5: AN INTERVENTION STUDY TO ASSESS THE EFFECT OF DRY COW THERAPY ON THE INCIDENCE OF CLINICAL MASTITIS IN THE SUBSEQUENT LACTATION IN SUCKLER EWES

5.1 ABSTRACT

An intervention study to assess the effect of dry cow therapy (DCT) (Ubro Red, Boehringer Ingelheim, Ingelheim, Germany) on the incidence of clinical mastitis cases in suckler ewes in the subsequent lactation was implemented on a convenience selected farm with an annual incidence of clinical mastitis of 5-10%. The hypothesis was that suckler ewes receiving broad spectrum intramammary antibiotic at weaning had a significantly ($p < 0.05$) lower risk of developing clinical mastitis in the subsequent lactation than ewes that did not receive this treatment. Approximately 50% of the ewes on the farm were randomly selected to receive DCT and 50% left untreated. Cases of clinical mastitis were recorded in the whole flock in the subsequent year. The incidence of clinical mastitis over one year was reduced by 70% in ewes treated with DCT; the incidence of clinical mastitis in early lactation was markedly lower in ewes receiving DCT.

5.2 INTRODUCTION

Clinical mastitis in suckler ewes is of welfare and financial concern to sheep farms (Winter, 2001). Morbidity or death of the ewe may result or a loss of functionality mammary gland or chronic pathological changes of the affected gland may result in the ewe being culled (Conington *et al.*, 2008). Clinical mastitis in ewes has been associated with lower lamb weights at weaning and higher mortality rates in lambs

(Arsenault *et al.,* 2008). Treatment of clinical mastitis in suckler ewes is reactive when a clinical case arises and typically comprises systemic or intramammary antibiotic treatment, with or without administration of an anti-inflammatory. In dairy ruminants, an intramammary antibiotic preparation, known as dry cow therapy (DCT) is frequently used in at drying off as a preventative measure, to reduce the incidence of mastitis over the dry period and subsequent lactation. However, DCT is rarely implemented in suckler ewes.

The aim of this study was to assess the effect of DCT on the incidence of clinical mastitis in a suckler ewe flock with a history of a high annual incidence of clinical mastitis.

5.3 MATERIALS AND METHODS

A minimum of 1204 ewes were required on a farm with an annual incidence of clinical mastitis of 8% to detect a 50% reduction in clinical mastitis cases in the subsequent lactation, where 50% of the ewes were randomly selected to receive DCT at weaning (power = 80%; $\alpha = 0.05$).

A commercial upland flock in Northumberland with 1400 ewes, with an annual incidence rate of clinical mastitis of 5%-10% was convenience selected as the study farm. At weaning 2010, each ewe was examined by the farmer to decide whether it would be retained in the flock for the next breeding year. Those with evidence of clinical mastitis, including abnormal palpable intramammary lumps, were identified for culling and excluded from the study.

There were 1282 ewes enrolled into the study at weaning in September 2010, 668 of which received treatment with DCT and 614 were left untreated. Treated and

untreated ewes were given different coloured ear tags. With the exception of the first day of the study, when the researcher visited the farm to teach the shepherd the protocol for udder examination and DCT administration, ewes were randomly selected and DCT was administered by the shepherd. The researcher recorded ewe age, body condition score (BCS) and the presence of teat lesions from all 132 ewes handled on day one of the study. Of these 132 ewes, 15 ewes in the non-treatment group and 16 ewes in the treatment group were randomly selected for udder half milk somatic cell counting before the DCT was administered, using the methods described in Chapter 2.

In ewes selected to receive treatment, a broad spectrum intramammary antibiotic treatment containing 100mg framycetin sulphate, 100mg penethamate hydriodide and 300mg procaine penicillin (Ubro Red, Boehringer Ingelheim) was aseptically administered into both udder halves using a whole tube for each udder half.

At lambing 2011 the researcher re-visited the farm. Thirty two ewes that had lambed in the previous 48 hours were convenience selected for milk somatic cell counting, 15 of these had received DCT in October 2010 and 17 had not received DCT. Milk samples were collected from both udder halves within the first 12-48 hours of lambing as previously described and ewe age, BCS and the presence of teat lesions also recorded.

Between September 2010 and September 2011, when the shepherd saw a case of clinical mastitis on the farm, the date, ewe ear tag, age, BCS, the udder half affected and the presence of teat lesions was recorded.

Blood samples were collected by the farm's private veterinary practitioner from a non-random selection of 20 study ewes that were poor condition score to assess flock

exposure to Maedi-Visna infection as part of a regional disease investigation. Blood samples were submitted to the Scottish Agricultural College (SAC) for Agar Gel Immunodiffusion Test (AGID) testing for Maedi-Visna antibody.

A partial farm budget (PFB) analysis to assess the financial viability of the use of DCT as a prevention measure for mastitis on this farm was conducted. This was calculated by subtracting the total benefits (B), including costs of disease avoided from the total costs (C) of the prevention of mastitis by use of DCT for the whole flock. Data obtained from the study on the incidence of clinical mastitis in ewes that did and did not receive treatment were used. Estimates of retail costs of Ubro Red DCT tubes and of the pharmaceutical costs of treating each ewe with clinical mastitis were obtained from internet searches (www.farmacy.co.uk, October 2011). The estimate of an average cost of a flock visit by a vet to the farm was obtained from the Society of Practicing Veterinary Surgeons (SPVS fee survey, 2011). Recent estimates of ewe replacement costs were obtain from published information from industry bodies; the English Beef and Lamb Executive (EBLEX) and Quality Meat Scotland (QMS), the levy bodies for sheep and beef farmers in England and Scotland respectively (EBLEX 2011; QMS 2009). Live weight lamb prices and gross margin per upland ewe were obtained from EBLEX online monthly reports from ADAS.

5.4 RESULTS

5.4.1 Study flock

Flock size decreased during the study from 1282 ewes enrolled at weaning to 1243 ewes at lambing to 1036 ewes at the end of the one-year study. The majority of losses were 190 old ewes, which were culled from the flock towards the end of the study. The remainder exited the study before lambing either due to failure to produce

at least one viable lamb or from ewe death. Of the 1243 ewes in the flock at lambing, 52.8% (n=656) received DCT.

The majority of ewes reared twins over the subsequent lactation. Ewe age, BCS and the presence of teat lesions for the subsets of ewes in which these variables were observed at weaning and lambing were similar across treatment groups (Table 5.1). Ewes that were milk sampled at lambing were not the same individual ewes that had been sampled at weaning although they were of similar BCS and age. However, the prevalence of teat lesions was higher in ewes at weaning 2010 (n=64, 48.4%) when compared with ewes at lambing 2011 (n=12, 37.5%).

Table 5.1 Summary statistics for ewes observed for body condition score, age and teat lesion prevalence at weaning and lambing

	No. of ewes observed	**BCS**			**Age at lambing (years)**			**Ewe had at least 1 teat lesion**	
		Median	n	%	Median	n	%	n	%
Weaning									
total	132	3	53	40.2	3	72	54.5	64	48.5
Non-DCT	24	3.5	15	62.5	3	15	62.5	12	50.0
DCT	108	3	48	44.4	3	57	52.8	52	48.1
Lambing									
total	32	3.5	7	21.9	3	10	31.3	12	37.5
		or 3	7	21.9					
Non-DCT	17	3	5	29.4	4	5	29.4	5	29.4
DCT	15	3	4	26.7	3	6	40.0	7	46.7

5.4.2 Comparison of treatment groups at weaning

There was no significant difference between the weaning mean $\log_{10}$ SCC of udder halves of ewes that received DCT compared with udder halves of ewes that did not receive DCT (non-paired t-test of two means with equal variances, $p = 0.84$, $df = 60$; Table 5.2). The arithmetic mean SCC at weaning across all 62 udder halves in both

treatment groups was 2, 706 x 10^3 cells/ ml, the mean log_{10} SCC was 5.91 and the geometric mean was 805 x 10^3 cells/ ml.

Table 5.2 Mean log_{10} SCCs of udder halves of ewes in treatment groups at weaning

Log_{10} SCC at weaning					95% CI	
No. udder halves		Mean	Std. Error	St Dev.	lower	Upper.
Untreated	30	5.81	0.11	0.61	5.59	6.04
Treated	32	5.99	0.14	0.80	5.71	6.28
Total	62	5.91	0.09	0.71	5.73	6.09
Difference		-0.18	0.18		-0.54	0.18
Difference: mean (untreated)-mean (treated) = -1.01					df=60	Pr(T>t)=0.84

There were 132 ewes enrolled on day one of the study from which BCS, ewe age and presence of teat lesion scars were recorded, including 31 ewes where milk was sampled, and 108 / 132 that received DCT. Although a greater proportion of ewes were selected for training purposes to receive treatment on the first day of enrolment, ewes in treatment groups for which data were recorded were very similar with respect to age, and prevalence of teat lesions scars (Table 5.1) although the median BCS of ewes in the untreated group was 3.5, higher than in the treated group which had a median BCS of 3. Fifteen of 24 (62.5%) ewes in the non-treatment group for which BCS was observed were in BCS 3.5 (Figure 5.1), 12 (50.0%) had at least one teat lesion scar at weaning and 15 (62.5%) were 3 years old (62.5%, n=15) at the subsequent lambing (Figure 5.2). Of the 108 ewes in the DCT treatment group for which BCS was observed at weaning, 48 (44.4%) were in BCS 3, 52 (48.1%) had at least one teat lesion scar and 57 (52.8%) were 3 years old at the subsequent lambing. The overall prevalence of teat lesion scars at weaning was 45.4% (64/132 ewes).

Figure 5.1 Body condition score of ewes by treatment group at weaning

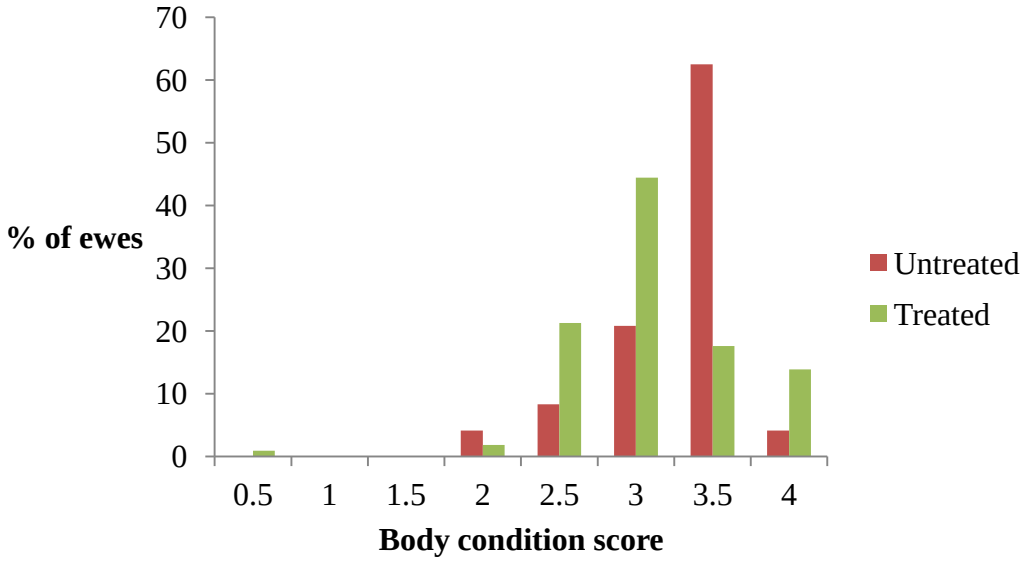
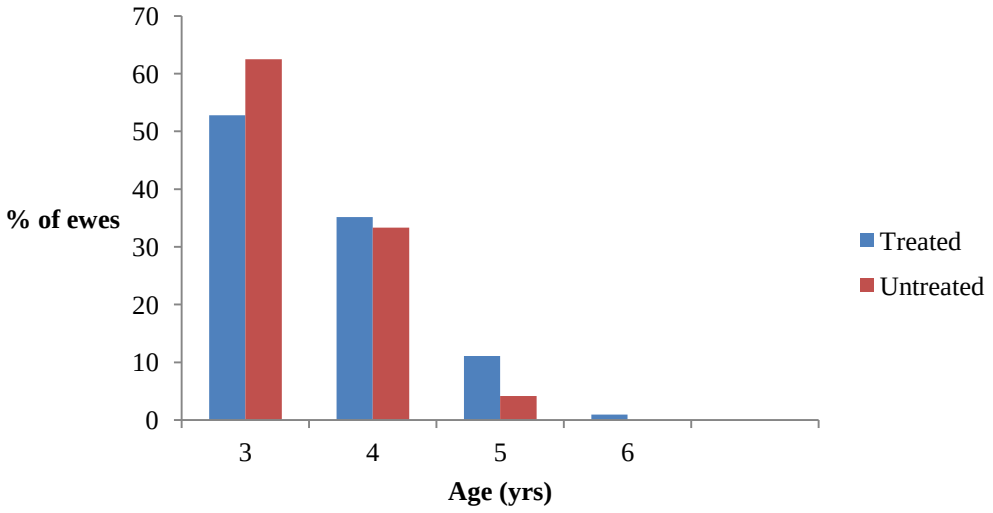


Figure 5.2 Age of ewes at subsequent lambing by treatment group at weaning



5.4.3 Effect of treatment on somatic cell count at lambing

Mean $\log_{10}$ HSCC at lambing was not significantly different ($p = 0.60$, $df = 60$) between the 17 ewes that did not receive DCT and 15 ewes that did receive DCT (Table 5.3). For both groups the mean $\log_{10}$ HSCC at weaning was significantly higher than at lambing ($p = 0.009$, $df = 60$) (Table 5.4).

Table 5.3 Mean log_{10} HSCC at lambing in sample of treated and untreated ewes

Log_{10} HSCC at lambing					95% CI	
	No. udder halves	Mean	Std.Err	Std. Dev	lower	upper
Untreated	34	5.48	0.09	0.50	5.31	5.65
Treated	30	5.54	0.05	0.26	5.44	5.64
Total	64	5.51	0.05	0.40	5.41	5.61
diff = mean (untreated)-mean(treated)		-0.061	0.101		-0.26	0.14
t=-0.60			Pr(T>t)=0.725			df=62

Table 5.4 Mean log_{10} HSCC at weaning and lambing in a sample of treated and untreated ewes

Log_{10} HSCC Untreated					95% CI	
	No. udder halves	Mean	Std.Err	Std.Dev	lower	upper
Weaning	30	5.81	0.11	0.61	5.59	6.04
Lambing	34	5.48	0.09	0.50	5.31	5.65
Total	64	5.64	0.07	0.57	5.49	5.78
diff=mean(weaning)-mean(lambing)		0.33	0.14		0.06	0.61
t=2.42			Pr(T>t)= 0.009			df=62

Log_{10} HSCC Treated					95% CI	
	No. udder halves	Mean	Std.Err	Std.Dev	lower	upper
Weaning	32	5.99	0.14	0.80	5.71	6.28
Lambing	30	5.54	0.05	0.26	5.44	5.64
Total	62	5.77	0.08	0.64	5.61	5.94
diff=mean(weaning)-mean(lambing)		0.46	0.15		0.15	0.76
t=2.99			Pr(T > t) = 0.002			df=60

Ewes that were milk sampled at lambing were very similar across treatment groups with respect to age, BCS and teat lesion scars (Table 5.1). Of the 17 ewes that had not received DCT, five (29.4 %) were in BCS 3 at lambing (there were fewer ewes in other BCS categories), and five (29.4%) had a teat lesion scar on at least one teat. Of the 15 of these ewes that had received DCT an equal proportion of ewes were in BCS 2.5, 3 or 3.5 (26.7%, n=4) at lambing and seven (36.8%) had a teat lesion scar on at least one teat. Of all 32 ewes observed at lambing, 12 (37.5%) had a teat lesion scar on at least one teat

5.4.4 Mastitis over one year observation period

Fifty cases of clinical mastitis were observed between enrolment in October 2010 and the end of the study in September 2011, an overall incidence of 3.9% over the one year observation period (Table 5.5). All cases were unilateral. The incidence of clinical mastitis over the observation period was significantly lower ($p< 1.0 \times 10^{-3}$, $z =4.06$) in treated ewes (n=12, 1.8%) compared with untreated ewes (n=38, 6.2%) a reduction of 70.9% (Table 5.5).

Table 5.5 New cases of clinical mastitis observed in untreated and treated ewes

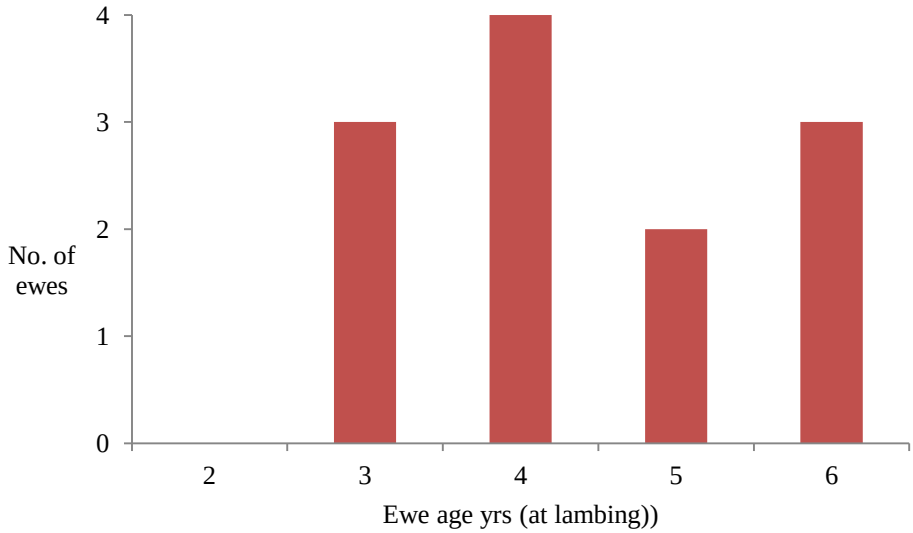
		Mastitis		
N obs (%)		No	Yes	Subtotal
Treated	No	576 (93.81)	38 (6.19)	614 (47.89)
	Yes	656 (98.20)	12 (1.80)	668 (52.11)
	Subtotal	1232 (96.10)	50 (3.90)	1282 (100.00)

The relative risk of clinical mastitis (where untreated ewes were considered exposed) was 3.45, where the risk of clinical mastitis in an untreated ewe was 0.062 and in a treated ewe was 0.018 through the year observation period.

5.4.5 Clinical mastitis in the dry period

All 12 cases (100%) of clinical mastitis observed in the dry period between weaning 2010 and lambing 2011 were in untreated ewes that were 3 to 6 years old at lambing (Figure 5.3).

Figure 5.3 Age distribution of ewes with clinical mastitis during the dry period



5.4.6 Mastitis in lactation

During lactation clinical mastitis was observed in 38 ewes, with 26 (66.7%) of these observed in untreated ewes and 12 in treated ewes (33.3%). Only one ewe in which clinical mastitis was observed over lactation was rearing a single lamb, three were rearing triplets and the remainder rearing twins (Table 5.6).

The temporal pattern of clinical mastitis over lactation was biphasic with a peak incidence of clinical mastitis in weeks 3 to 4 of lactation (Figure 5.4) and few observations of mastitis between weeks 9 and 12 of lactation.

There were more cases of mastitis in the first half of lactation (wks 0-9) in ewes that had not received DCT (n=19) than in ewes that had received DCT (n=5); untreated ewes had a peak in mastitis incidence in weeks 3 to 5 in lactation whilst there was no peak of cases observed in treated ewes. There were the same number of new mastitis cases in treated ewes (n=7) and untreated ewes (n=7) in the second half of lactation (Figure 5.5).

The incidence rate of clinical mastitis in untreated (exposed ewes) was 0.012 per ewe month in lactation, whilst that of treated ewes was 3.6 x 10^{-3} per ewe month in lactation over five months of lactation. The incidence rate ratio of mastitis in untreated to treated ewes was 3.45.

Figure 5.4 The number of ewes with clinical mastitis by weeks in lactation

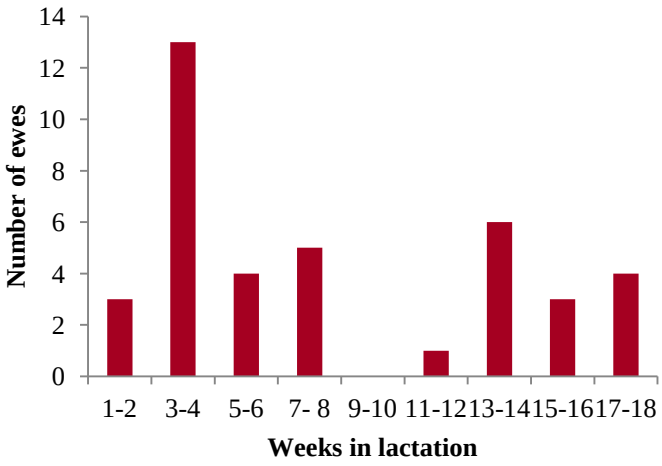
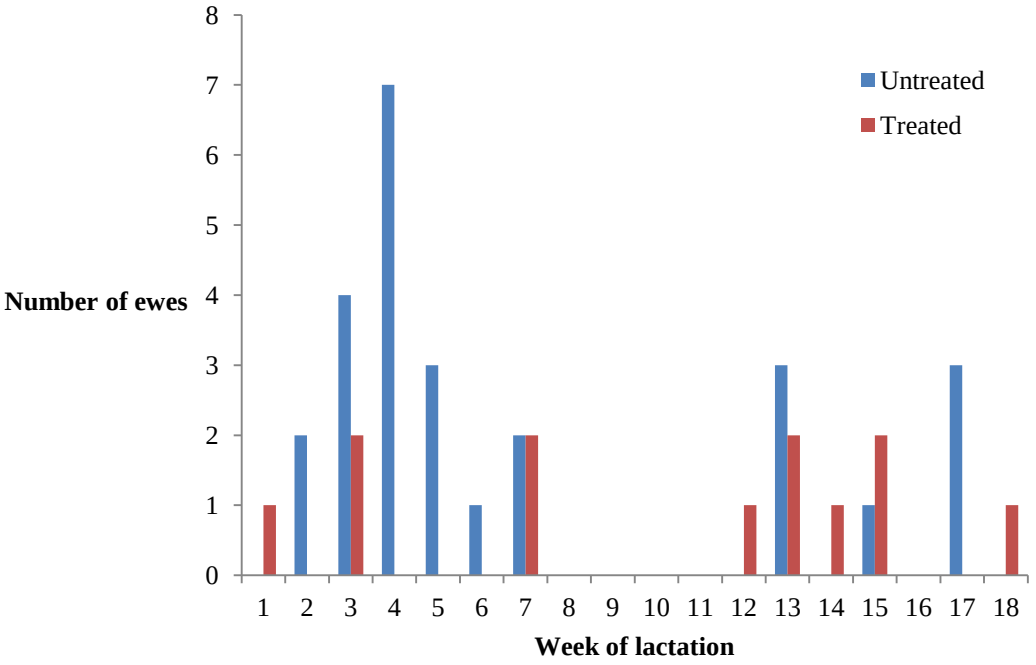


Table 5.6 Summary statistics for categorical variables for clinical mastitis cases during lactation

	Untreated			Treated		
Categorical variables	n obs	N	% obs	n obs	N	% obs
Age at lambing (years)						
1	1	26	3.85	0	12	0.00
2	6	26	23.08	0	12	0.00
3	3	26	11.54	6	12	50.00
4	6	26	23.08	6	12	50.00
5	5	26	19.23	0	12	0.00
6	5	26	19.23	0	12	0.00
BCS at time of mastitis						0.00
1.5 or less	2	26	7.69	0	12	0.00
2	4	26	15.38	0	12	0.00
2.5	5	26	19.23	3	12	25.00
3	11	26	42.31	5	12	41.67
3.5	3	26	11.54	4	12	33.33
4 or more	1	26	3.85	0	12	0.00
Ewe had a traumatic or non-traumatic teat lesion at mastitis	20	26	76.92	5	12	41.67
Udder half had a traumatic or a non-traumatic teat lesion at mastitis	19	26	73.08	5	12	41.67

Figure 5.5 New cases of ewe mastitis in treatment groups by week of lactation



Clinical mastitis was observed in treated ewes in good BCS whereas in untreated ewes, clinical mastitis was observed across all body condition scores (Table 5.6).

5.4.7 Teat lesions

In total, 218 udder halves of 195 ewes were examined for teat lesions at weaning, lambing or as a case of clinical mastitis. Of these, 174 ewes were examined on only one occasion, whilst 23 observations were from 21 ewes examined on more than one occasion (19 observed twice, two observed three times). One hundred and one (51.8%) ewes had at least one teat lesion on a teat at an observation and of these a similar percentage of ewes had a teat lesion on one teat (42.3%, n=50; 46 ewes observed once and two ewes observed on two occasions) as on both teats (47.2%, n=51). There were 94 ewes (48.2%) that had no teat lesions; seven of these ewes were observed more than once (five ewes which were observed on two occasions and two ewes which were observed on three occasions). The number of observations of teat lesions in right and left udder halves was approximately equal; 78 on the left

udder half (57 scars, eight fresh and 16 unspecified), and 74 ewes on the right udder half (46 scars, eight fresh, and 20 unspecified).

Teat lesions were observed on 25 (65.8%) of 38 affected udder halves of ewes observed with clinical mastitis during lactation. A significantly higher proportion ($p < 0.03$) of ewes that had not received DCT had teat lesions on the affected udder half (80%, n=20) than those that had received DCT (20%, n=5) (Table 5.7), although approximately equal proportions of ewes in each treatment group had teat lesion scars at weaning. Teat lesion scars were not observed on any of the 12 udder halves of the 12 ewes which developed clinical mastitis during the dry period.

Table 5.7 Teat lesions in untreated and treated ewes with mastitis in lactation

	Ewe with mastitis had teat lesion		
	No	Yes	Total
no DCT	6	20	26
DCT	7	5	12
Total	13	25	38
Pearson χ^2=4.53	df=1		Pr > χ^2= 0.033

5.4.8 Maedi Visna virus exposure

Fourteen out of 20 (70%) ewes tested were seropositive for Maedi Visna virus antibodies.

5.4.9 Partial farm budget

It was estimated that, with a reduction in clinical mastitis incidence of 70.9% with the use of DCT and with the assumptions listed (Table 5.8), the costs of implementing a prevention plan using DCT would outweigh the benefits at a loss of £34.30 per £100 ewes. With the same assumptions, the price of DCT would need to be less than £3.60 per ewe (£1.83 per tube) to make preventive DCT cost effective. At a price of £2 per tube and with all other assumptions remaining unchanged (Table

5.8) assuming the same reduction in incidence of clinical mastitis with treatment, the annual incidence of clinical mastitis on the farm would need to be at least 6.89% before the financial benefits of DCT outweighed the financial costs.

Table 5.8 Partial farm budget for prevention of mastitis using dry cow therapy

Costs and benefits for whole flock DCT use per 100 ewes					
	Cost per unit	**Refs**	**Key**	**Calculation**	**£**
Control Costs (C)					
Ubro Red	£4 per ewe	Farmacy.co.uk, 2011	a	4 x 100	400.00
Vets time	£ 50 for visit and consult	SPVS, 2011	b	50 x 1	50.00
Famers time	£8.20/hr	DEFRA, 2011	c	4 x 100 x (8.2/60)	54.70
Cull ewe price not received*	£78 per ewe	QMS, 2009	d	3.4 x 78	265.00
Transport costs	negligible		e		0.00
subtotal					769.70
Control Benefits (B) (including costs of disease avoided)					
Mortality prevented	£47 per ewe	QMS, 2009	f	1 x 4.7	47.00
Vet costs saved	£ 50 per visit	SPVS, 2011	g	1 x 30	50.00
Treatment costs saved	£0.70 per ewe		h	0.7 x 4.4	3.08
Farmer time saved	£6.55 per ewe	DEFRA, 2011	i	4.4 x (8.2/60) x 45	27.06
Lamb mortality avoided	£100 per lamb	EBLEX, 2011	j	4.4 x (0.8 x 1.6) x 100	563.20
Suboptimal weight gain avoided	£1.94/kg	EBLEX, 2012; Laarsgard *et al.*,1993	k	4.4 x (0.8 x 1.6) x 1.94	10.93
Lower feed costs	negligible		l		0
Ewe replacement costs	12.30 per ewe	EBLEX, 2011	m	4.4 x 12.3	54.12
subtotal					735.4
Costs of control (C) - benefits of control (B) (£ per 100 ewes)					34.30

Key	**Assumptions** (Per 100 ewes unless otherwise stated)
a	Assumes each tube costs £2 and 2 used per ewe
b	Assumes per farm to discuss mastitis prevention (excluding pharmaceuticals)
c	Assumes farmer labour costs are £8.20 per hour. Assume 4 mins extra per ewe at drying off check

d	Assumes 3.4 ewes not culled (assume 1 dies)
e	Assumes negligible change in transport costs
f	Assumes gross margin of 1 upland ewe death averted
g	Assumes that the vet normally does one visit per year in reaction to a mastitis case but is not called out with a lower incidence
h	Assumes antibiotic treatment cost of 1 injection of BetamoxLA
i	Assumes 45mins per case for treatment and mothering on of lambs (may be spread over several days)
j	Assumes 1.6 lambs expected to be reared per ewe without mastitis but probability of lamb survival if ewe has mastitis is decreased by 20%
k	Assumes lambs reared by ewes without mastitis weigh 4kg more at weaning
l	Assumes negligible change in feed costs
m	Assumes 4.4 breeding ewes are replaced in next season

*Assuming a famer would send ewes with mastitis that survived to the abattoir.

5.5 DISCUSSION

5.5.1 Effect of dry cow therapy on clinical mastitis incidence

In this study flock, treatment with a broad-spectrum dry cow therapy (DCT) at drying-off was significantly associated with a decreased risk of clinical mastitis in the subsequent lactation.

Dry cow therapy reduced the incidence of clinical mastitis over one year by 70%, from 6.2% to 1.8% and particularly reduced the number of cases in early lactation and dry period. Results from this study suggested at least 75% of clinical mastitis cases on this farm were from intramammary infections that were acquired in or persisted through the dry period, assuming DCT was 100% effective at removing these infections. Dry cow therapy was most effective for preventing clinical mastitis during the dry period and in the first half of lactation; almost four times the number of clinical mastitis cases observed in the first half of lactation were in ewes that had not received DCT compared with ewes that had received DCT and all cases recorded in the dry period were in untreated ewes. This suggests that DCT may be most effective in reducing clinical mastitis cases in early rather than late lactation and that

the duration of effect of DCT is mostly in the first half of lactation. However, it is not possible to determine, whether clinical cases observed in lactation were caused by pathogens acquired in lactation or whether this was because of incomplete clearance of bacteria by the DCT.

Bacteriological analysis of milk samples was out with the scope of this study thus whether the effect of DCT was pathogen dependent is not known. Indeed whether new clinical cases in lactation were caused by any particular pathogens that commonly cause clinical mastitis, such as *Mannheimia haemolytica* or *Staphylococcus aureus* was not investigated in this study. The dry-off treatment used was a broad spectrum treatment previously demonstrated to be effective in removing some Gram negative and Gram positive pathogens (Bradley and Green, 2001). Based on this spectrum of action in dairy cows, it is likely that dry period infections with these pathogens would have been eradicated in sheep. Furthermore Bradley and Green (2001) demonstrated that this DCT was more effective in the prevention of *E. coli* mastitis in the first 100 days of lactation in dairy cows than a narrow spectrum DCT with no Gram negative efficacy, and framycetin has been demonstrated to have particularly high (98%) efficacy *in vitro* against *E. coli* and Gram negative isolates from dairy cow mastitis (Menzies *et al.*, 2000).

There is a theory that removal of minor pathogens by the use of DCT may actually predispose to susceptibility to clinical disease from subsequent infections with major pathogens. That the eradication of dry period infections caused by minor pathogens may predispose to clinical mastitis caused by major pathogens has been previously demonstrated with *E. coli* mastitis in dairy cows (Green *et al.*, 2002). There was no evidence for this from the current study, however. Work is necessary to further

investigate a protective role that minor pathogens play could play in suckler ewes, for example by comparing longitudinal patterns of bacterial IMI and clinical mastitis before and after the dry period and through lactation in groups of ewes that receiving broad spectrum DCT, narrow spectrum DCT and no DCT respectively.

It is difficult to make robust conclusions on whether the effect of treatment was dependent on ewe age or BCS. Another possible explanation is that these results merely reflect the age structure of the flock and that there were proportionately more of older ewes given DCT. This is plausible as ewes in treatment groups observed at weaning and lambing were also mainly of these BCS and age categories. However the observation of ewes in other ages with clinical mastitis in the dry period and in lactation, that had not received treatment, would make this less likely. Another explanation for the age distribution of clinical mastitis cases in treated ewes is that there was treatment bias towards ewes that were in good BCS and of the most common age groups. This seems unlikely given the instructions given and the high compliance and diligence or the shepherd in the other aspects of the study.

A high proportion of ewes tested were seropositive for Maedi Visna, indicating that this viral infection was circulating within the flock. Maedi Visna virus is known to be associated with udder pathology (Houwers and van der Molen., 1987), described as a chronic and indurative mastitis (van der Molen., 1985;) or exhibited by a hard udder (Pepin *et al.*, 1998). It is possible that Maedi Visna virus infection may have altered the susceptibility to bacterial mastitis and the effects of treatment. The incidence of mastitis in untreated ewes in the year of study was consistent with that observed in a typical year for this farm which had a historical incidence of 5-10% per year. Bacteriological analysis was not conducted on milk samples that were collected so it

is therefore unknown whether the annual high incidence of clinical mastitis on this farm was associated with any particular pathogens.

5.5.2 Effect on dry cow therapy on teat lesions

A smaller proportion of treated ewes with clinical mastitis had teat lesions (20%) compared with untreated ewes with clinical mastitis (80%). This may suggest not only that DCT reduced the risk of developing teat lesions in the subsequent lactation but also that teat lesions are directly or indirectly associated with the risk of developing clinical mastitis. Work described in Chapter 3 revealed a negative association with teat lesions and lamb weight. In the same study, Cooper (Master's thesis, 2011) demonstrated that higher lamb weights were a risk factor for traumatic teat lesions (bites, tears or chapping). A reduced milk yield from ewes with subclinical IMI has been demonstrated by other authors **(**Albenzio *et al.*, 2002**)**. It is possible that milk yield was improved in those ewes that received DCT in our study and that this resulted in greater milk availability for lambs, thus aggressive nursing behaviour was reduced. Less aggressive nursing would not only result in a lower incidence of teat trauma but would also reduce butting behaviour that may increase the likelihood of rupture of walled-off microabscesses or sites of infection in the mammary parenchyma. Ewes that did not receive DCT would therefore have been at a greater risk of developing clinical mastitis and teat lesions. However, there was observation bias as only ewes with clinical mastitis were observed for teat lesions during lactation so incidence of teat lesions in non mastitic ewes over the lactation cannot be compared. Little differentiation was made for teat lesion type thus whether teat lesions in untreated ewes with mastitis were more likely to be fresh could not be investigated. Teat lesion prevalence at weaning and at lambing was very similar between untreated and treated groups thus teat lesions of treated and untreated

populations were similar and comparable before the lactation period. Teat lesion prevalence was slightly lower at lambing than at weaning in both groups, which is an expected finding since the visibility of teat lesions wane with time after weaning, when lambs cease to suckle.

5.5.3 Effect of dry cow therapy on somatic cell count

In the small number of ewes studied, DCT did not significantly affect mean $\log_{10}$ HSCC. In a study of the effect of using the same DCT on bulk milk SCC of dairy flocks in Spain, bulk milk tank SCC of flocks was significantly lower in the subsequent lactation compared to the lactation before dry off in 23 treated machine milked flocks (selected for bulk milk tank SCC >1 x 10^6 cells/ml). However, no difference between lactation SCC (pre-treatment) and SCC in the subsequent lactation was observed in two treated hand milked flocks in the study although dry off SCC was significantly higher in hand milked ewes than in machine milked ewes (Gonzalo *et al.*, 2009). This suggests that management differences between types of flocks may result in poorer hygiene and an associated higher prevalence of IMI in hand milked flocks and that DCT may not be as effective at removing IMI in flocks where management practices are less hygienic. Management practices of suckler sheep may also be associated with a higher prevalence of intramammary infections compared to machine milked dairy sheep, which may account for the lack of effect on mean $\log_{10}$ HSCC in our study flock.

5.5.4 Partial farm budget

The partial farm budget performed provided some indication of the financial practicalities and benefits of implementing such an intervention at the current costs used, and assuming all other variables including clinical mastitis incidence in ewes that do and ewes that do not receive DCT, implementing DCT would result in the

farmer making a small loss (£34.30 per 100 ewes). However, estimates of variables included in the PFB model such as lamb live weight and ewe cull prices, ewe replacement prices are highly variable year on year depending on market forces. Furthermore, farm labour and veterinary costs may be different according to region and scale of enterprise. As such the figures should be interpreted with caution. Although at the current price of DCT used in the PFB, the costs of the use of DCT were higher than the financial benefits for the commercial flock studied, farms with ewes of a particularly high value, for example pedigree flocks, may find this a cost-effective intervention where clinical mastitis incidence is sufficiently high.

5.5.5 Limitations of study

Although this study demonstrates that this DCT preparation did significantly reduce the incidence of clinical mastitis on this farm, particularly those in the first month of lactation, the study is limited in its potential for these results being extrapolated to other flocks. As this study was only a case study of one farm, there were a low number of ewes with mastitis overall to enable robust conclusions to be drawn across suckler flocks with a high incidence of clinical mastitis. A logistic regression model could not be constructed to assess the effect of variables such as ewe age, BCS and teat lesions on the incidence of clinical mastitis due to the low number of observations of these variables in ewes without mastitis during lactation; observations of these variables of ewes without mastitis during lactation were only made at lambing, and through the rest of lactation only of ewes with mastitis. The randomised controlled design of this study allows robust comparisons to be drawn and the author is reasonably confident that randomisation of treatment and sampling was done as far as could be practically implemented. Treated and untreated ewes from which more detailed observations were recorded at weaning and lambing were

conveniently sampled with respect to time the author was present or the shepherd was able to make these observations but ewes were not managed according to any particular age or other management structure and there was no other known selection bias. No differences were found between these treatment groups at either weaning or lambing and thus they were considered representative of their respective treatment group.

The findings in this study indicate that further work is necessary to investigate the effect of DCT on SCC in flocks with high levels of clinical mastitis. Concurrent bacteriological analysis and somatic cell counting of milk collected from treated and untreated ewes before and after the dry period as well through lactation from a larger number of ewes and across several flocks would enable a more robust comparison of the effect of DCT on IMI and provide scope to investigate whether any effects are pathogen dependent.

5.6 CONCLUSIONS

The administration of a broad spectrum antibiotic treatment at drying off was beneficial in reducing the incidence of clinical mastitis by 70.9% on a farm with a previous annual incidence of clinical mastitis 5%-10%. A partial farm analysis did not find this to be a cost effective intervention in the year of study where the incidence of clinical mastitis in untreated ewes was 6.2%.

5.7 ACKNOWLEDGEMENTS

The provision of the Ubro Red intramammary tubes used in this intervention study free of charge by Boehringer Ingelheim is gratefully acknowledged.

6. CHAPTER 6: GENERAL DISCUSSION

There were a number of novel findings from the four studies, most notably the associations between udder conformation, intramammary infection (IMI), teat lesions and lamb growth. This demonstrated the importance of overall udder health for optimising production of suckler ewes.

The initial study was the first study to characterise the longitudinal pattern of udder half somatic cell count (HSCC) in suckler ewes and describe HSCC variation with days in lactation (DIL), DIL^2 and DIL^3. Knowledge of the longitudinal pattern of SCC that may be expected in suckler ewes is of importance so that SCC may be robustly be used in research as a tool to monitor IMI. The majority of milk samples cultured yielded bacterial growth and high values of SCC were observed across all of studies. We can therefore conclude that IMI is very common in UK suckler ewes. Higher SCC was observed in ewes that were old, thin, had a greater drop of the suspended udder or larger teats; these variables may thus be considered risk factors for IMI. Older ewes are likely to have higher levels of infection as they been exposed to a greater number of infections over previous lactations and therefore may be more likely to harbour existing chronic disease. Thinner ewes that are metabolically stressed may be more likely to succumb to new infections.

The importance of udder health on lamb production was demonstrated. When controlling for birth weight of lamb, age of lamb, being reared in a multiple litter and diarrhoea in the lamb, significantly lower lamb weights were observed when the ewe had a mean SCC>400,000 cells/ml or had had a traumatic teat lesion (bites, tears or chapping) two weeks previously. Lambs reared by a ewe that was in body condition score (BCS) of 2.5 or less before lambing weighed significantly less than those

reared by ewes in BCS of 3 or more. This is the first study to simultaneously assess the effect of all of these variables, to demonstrate an association between udder conformation and IMI in suckler ewes and describe a cut off of SCC above which lower lamb weights are observed.

The effect of broad-spectrum dry cow therapy (DCT) on subsequent subclinical and clinical mastitis was investigated for the first time in suckler ewes. Dry cow therapy was of benefit in reducing the incidence of clinical mastitis on a farm with a high incidence of clinical mastitis. However, on a farm with a low incidence of clinical mastitis, the use of DCT offered no apparent benefit and did not significantly reduce the level of SCC in the subsequent lactation or result in increased lamb weight. This may have been because DCT did not reduce new or existing IMI on this farm, as suggested by limited reduction in SCC, or there was no association between SCC and weight in this study. The use of DCT to reduce the incidence of clinical mastitis in the next lactation could be considered by farms with a high incidence of clinical mastitis. However, this is unlikely to be financially viable on commercial flocks given current variable cost estimates, although it may be a more cost effective intervention for pedigree flocks.

The findings from the four studies also make a considerable contribution to the knowledge of IMI in suckler ewes that are pertinent to industry as they provide hypotheses that can be tested to suggest some simple on farm interventions to improve flock udder health and productivity. One hypothesis that could be tested is whether appropriate feeding of ewes through late gestation and lactation reduces the risk of IMI whilst optimising lamb growth. This could be assessed using a matched cohort study to investigate the associations between BCS and diet and BCS and SCC,

lamb weight and occurrence of teat lesions. Another hypothesis that could be tested is that removal of ewes that are old, persistently thin despite good feeding or have poor udder conformation before the next breeding cycle, and selection against ewes with poor udder conformation when breeding ewes for replacements reduces IMI.

Each of the four studies in the current thesis would have been enhanced by the investigation of the bacterial species present in each milk sample collected, using more robust bacterial identification techniques than the one plate culture technique employed. However, given the budgetary and time constraints, no bacteriology was conducted for three of the four studies. In the first study, the only study where bacteriology was performed, identification of bacterial species at the strain level using molecular techniques would have provided more information on how IMIs persist or recrudesce, further contributing to our knowledge of longitudinal patterns of intramammary infections, and the role of different udder microflora. In the second study, bacteria species present in each milk sample would have provided information for assessment of whether udder conformation and teat lesions are risk factors for the presence and persistence of particular bacterial species. In the third and fourth studies, the effect of DCT on removal and speed of re-infection or recolonisation of different species of bacteria could have been assessed if bacteriology had been performed on each milk sample. Some milk samples have been stored and future work includes investigation of the bacteria in these samples.

This thesis has identified the importance of udder health on production and identified hypotheses for routes to improve udder health. Future work should focus further on the role of improved udder and teat conformation to reduce intramammary infections. In addition, the identification of longitudinal patterns of bacterial flora of

the udder skin compared to that inside the udder in ewes with and ewes without teat lesions would allow potential identification of the longitudinal relationships between external and internal flora of the udder. Another key area for further investigation is the persistence of bacteria over the dry period and across lactations; molecular techniques applied to milk collected before and after the dry period and in subsequent lactations could be used to identify which species and strains of bacteria are present to provide more information on how IMI is cleared or persists.

In conclusion, the results from these studies have contributed vastly to our knowledge of longitudinal patterns of IMI in suckler ewes and generated hypotheses for future studies to reduce the incidence of IMI and increase productivity of suckler ewes.

7. APPENDICES

7.1 Chapter 2 Appendices

Figure A.2.1 Fit to Normal distribution of log_{10} SCC for all observations

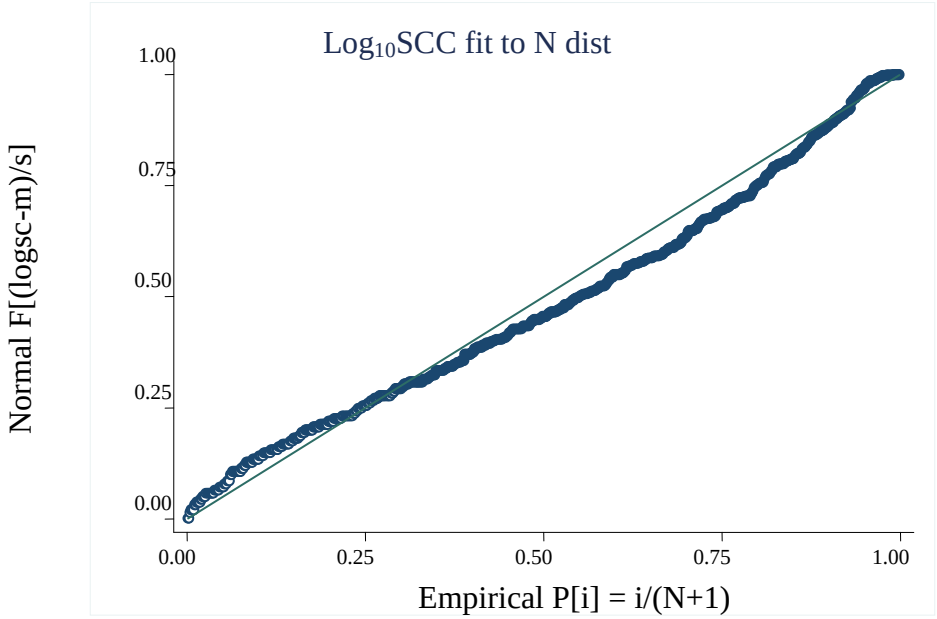


Table A.2.1 Repeatability of blinded bacterial identification using one plate culture technique of matched milk samples

Absence (0) or Presence (1) of bacteria typed identify in matched samples independently assessed

CNS

	Batch B		
Batch A	Absent	Present	Total
Absent	6	3	9
Present	7	23	30
Total	13	26	39

Bacillus

	Batch B		
Batch A	Absent	Present	Total
Absent	26	6	32
Present	3	4	7
Total	29	10	39

Gram neg

	Batch B		
Batch A	Absent	Present	Total
Absent	26	4	30
Present	3	6	9
Total	29	10	39

Corynes

	Batch B		
Batch A	Absent	Present	Total
Absent	38	0	38
Present	0	1	1
Total	38	1	39

Nocardia

	Batch B		
Batch A	Absent	Present	Total
Absent	21	2	23
Present	6	10	16
Total	27	12	39

Staph aureus

	Batch B		
Batch A	Absent	Present	Total
Absent	36	0	36
Present	1	2	3
Total	37	2	39

Coliforms

	Batch B		
Batch A	Absent	Present	Total
Absent	39	0	39
Present	0	0	0
Total	39	0	39

Mannheimia

	Batch B		
Batch A	Absent	Present	Total
Absent	39	0	39
Present	0	0	0
Total	39	0	39

Proteus

	Batch B		
Batch A	Absent	Present	Total
0	39	0	39
1	0	0	0
Total	39	0	39

Streptococcus

	Batch B		
Batch A	Absent	Present	Total
0	38	0	38
1	0	1	1
Total	38	1	39

No Growth

	Batch B		
Batch A	Growth	No Growth	Total
Growth	34	4	38
No Growth	0	1	1
Total	34	5	39

Contaminated

	Batch B		
Batch A	Uncontaminated	Contaminated	Total
0	38	0	38
1	0	1	1
Total	38	1	39

Table A.2.2 Testing for test bias for blinded bacterial identification using one plate culture technique of matched milk samples

Bacteria type	McNemars χ^2 test statistic (1.d.f)	$P > \chi^2$	Exact McNemars significance probability
CNS	1.60	0.21	0.34
Bacillus	1.00	0.32	0.51
GramNegativeA	0.14	0.71	1.00
Corynes	n/a	n/a	1.00
Nocardia	2.00	0.16	0.29
Staphaureus	1.00	0.32	1.00
Coliforms	n/a	n/a	1.00
Pasteurella	n/a	n/a	1.00
Proteus	n/a	n/a	1.00
Strep	n/a	n/a	1.00
Contaminated	n/a	n/a	1.00
No Growth	4.00	0.05	0.125

The null hypothesis is that there is no significant difference in the rate at which which the test show disagreement (test bias).There is no evidence that test bias exists, therefore we can assume we can use Kappa as an evaluation of test agreement.

Table A.2.3 Testing for agreement of blinded bacterial identification using one plate culture technique of matched milk samples

Bacteria type	**Agreement (%)**	**Expected agreement (%)**	**Kappa**	**95% CI**		**Z**	**P>Z**
				lower	upper		
CNS	74.36	58.97	0.38	0.07	0.68	2.42	0.01
Bacillus	76.92	65.61	0.33	0.02	0.63	2.11	0.02
Gram neg	82.05	63.12	0.51	0.20	0.83	3.21	0.00
Corynes	100.00	95.00	1.00	0.69	1.31	6.24	0.00
Nocardia	79.49	53.45	0.56	0.25	0.87	3.58	0.00
Staph aureus	97.44	87.97	0.79	0.48	1.09	5.03	0.00
Coliforms	100.00	0.00	n/a				
Pasteurella	100.00	0.00	n/a				
Proteus	100.00	0.00	n/a				
Strep	100.00	0.00	n/a				
Contaminated	100.00	95.00	1.00	0.69	1.31	6.24	0.00
No Growth	89.74	85.27	0.30	0.08	0.53	2.64	0.00

For all bacteria types, and for classing the sample as contaminated, of not having any bacterial growth, the level of agreement is significantly better than expected due to

chance although there are wide confidence intervals about each estimate of Kappa. We can say there is fair agreement between batches for CNS, *Bacillus* and no growth, moderate agreement for Gram negatives and *Nocardia*, substantial agreement for *S. aureus* and No growth, and almost perfect agreement for *Corynebacteria* and contamination. However there is a moderately high degree of uncertainty about these statements.

Figure A.2.2 Number of bacteria species isolated by age of ewe

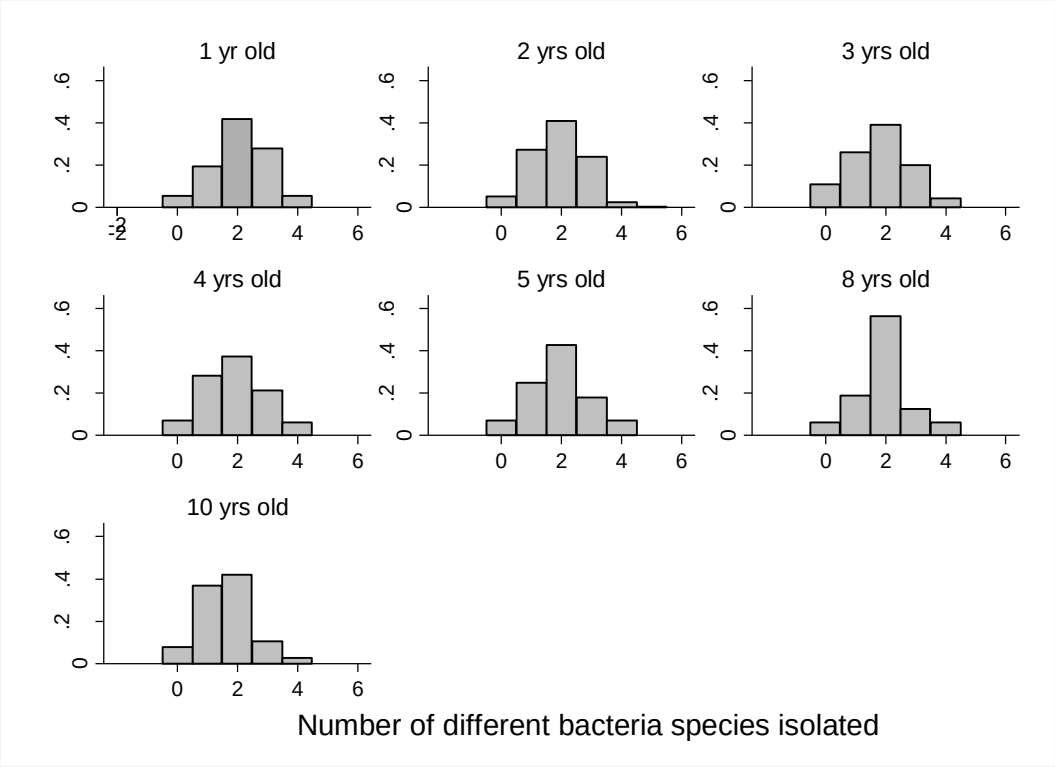


Figure A.2.3 Number of bacterial species isolated by week in lactation

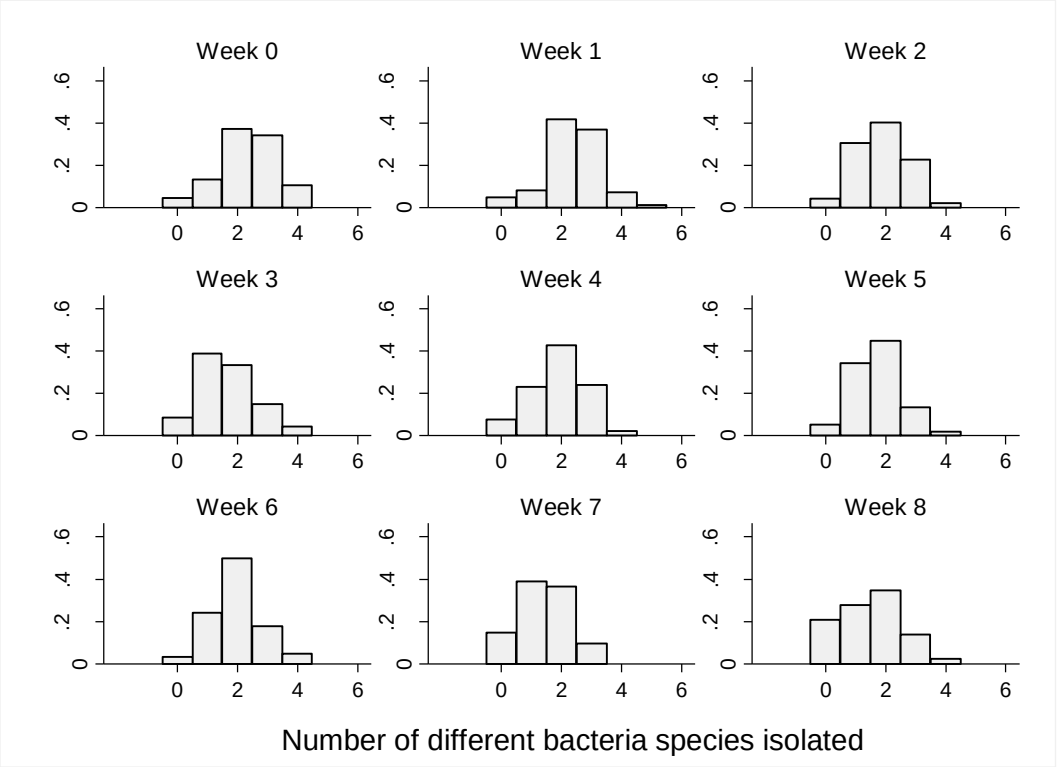


Table A.2.4 Bacteria isolated by age of ewe and by week in lactation

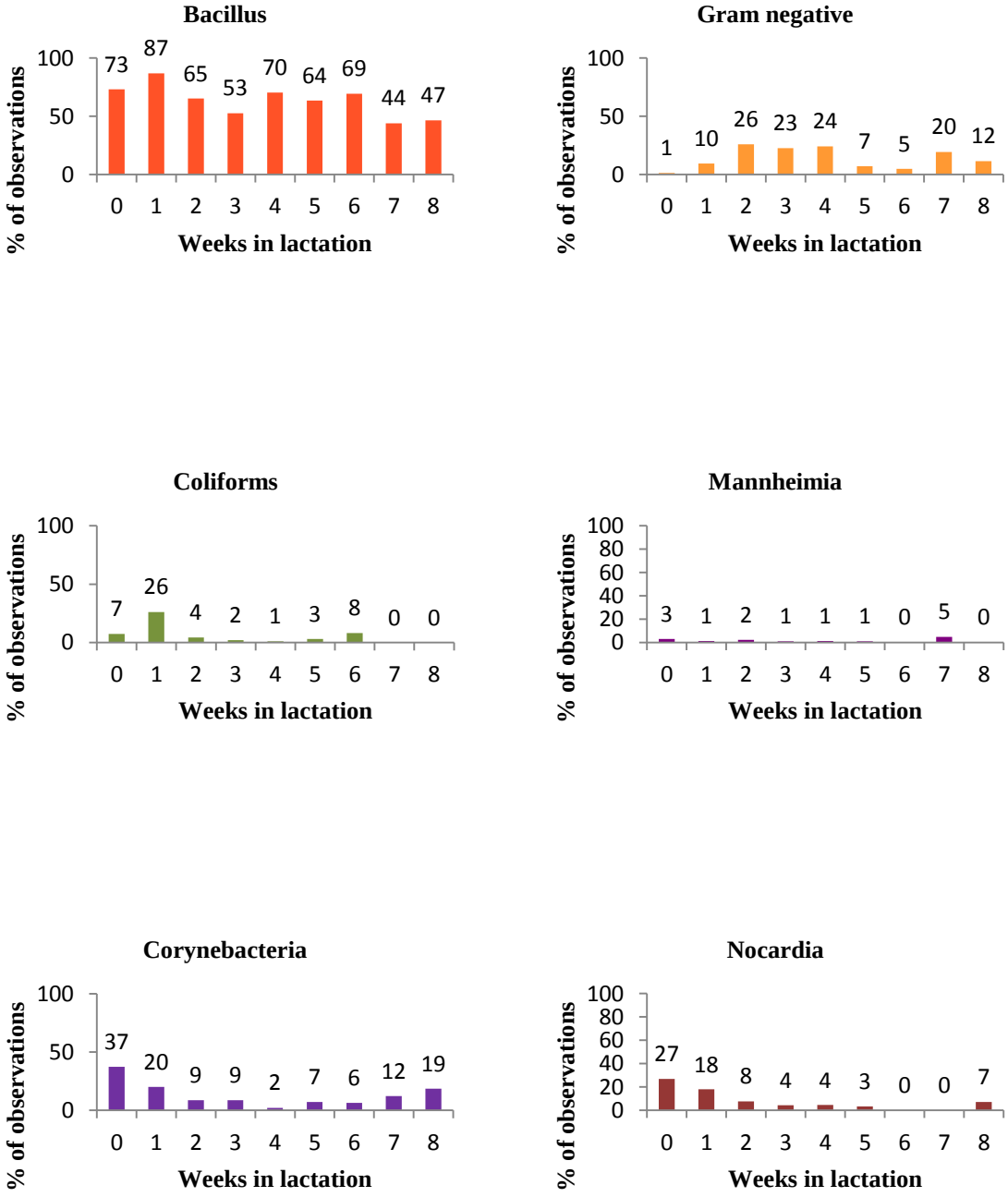
		Bacteria					
		Bacillus spp.		Coliform bacteria		Corynebacterium spp.	
Age of ewe	N observations	n	% of obs	n	% of obs	n	% of obs
1	72	51	70.83	7	9.72	8	11.11
2	250	174	69.60	16	6.40	30	12.00
3	166	98	59.04	12	7.23	23	13.86
4	99	65	65.66	4	4.04	14	14.14
5	28	19	67.86	1	3.57	3	10.71
8	16	6	37.50	2	12.50	3	18.75
10	38	24	63.16	0	0.00	3	7.89
all ages	669	437	65.32	42	6.28	84	12.56
Week of lactation	N observations	n	% of obs	n	% of obs	n	% of obs
0	67	49	73.13	5	7.46	25	37.31
1	84	73	86.90	22	26.19	17	20.24
2	92	60	65.22	4	4.35	8	8.70
3	93	49	52.69	2	2.15	8	8.60
4	91	64	70.33	1	1.10	2	2.20
5	96	61	63.54	3	3.13	7	7.29
6	62	43	69.35	5	8.06	4	6.45
7	41	18	43.90	0	0.00	5	12.20
8	43	20	46.51	0	0.00	8	18.60
all weeks	669	437	65.32	42	6.28	84	12.56

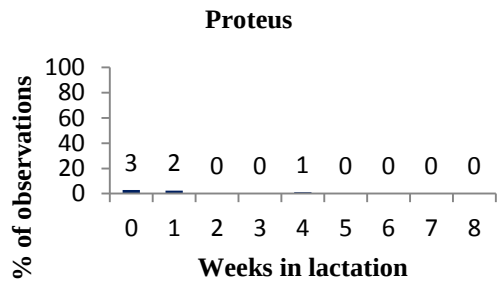
		Bacteria					
		Gram-negative bacteria		Mannheimia spp.		Proteus spp.	
Age of ewe	N observations	n	% of obs	n	% of obs	n	% of obs
1	72	16	22.22	0	0.00	3	4.17
2	250	39	15.60	0	0.00	0	0.00
3	166	21	12.65	0	0.00	1	0.60
4	99	14	14.14	5	5.05	0	0.00
5	28	6	21.43	0	0.00	0	0.00
8	16	0	0.00	5	31.25	0	0.00
10	38	3	7.89	0	0.00	1	2.63
all ages	669	99	14.80	10	1.49	5	0.75

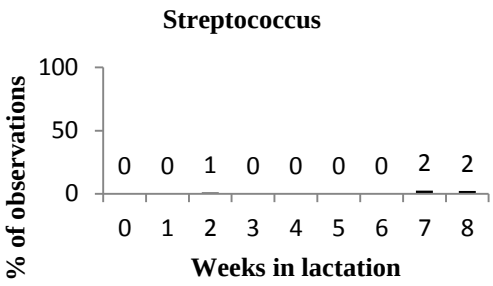
Week of lactation	N observations	n	% of obs	n	% of obs	n	% of obs
0	67	1	1.49	2	2.99	2	2.99
1	84	8	9.52	1	1.19	2	2.38
2	92	24	26.09	2	2.17	0	0.00
3	93	21	22.58	1	1.08	0	0.00
4	91	22	24.18	1	1.10	1	1.10
5	96	7	7.29	1	1.04	0	0.00
6	62	3	4.84	0	0.00	0	0.00
7	41	8	19.51	2	4.88	0	0.00
8	43	5	11.63	0	0.00	0	0.00
all weeks	669	99	14.80	10	1.49	5	0.75

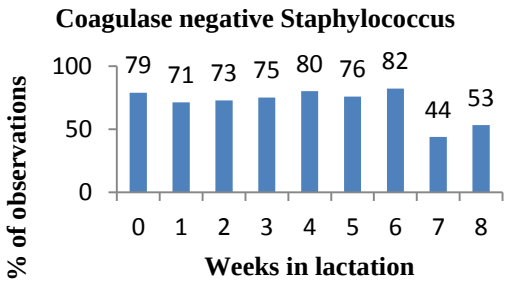
		Bacteria					
		CNS spp.		Staphylococcus aureus		Streptococcus spp.	
Age of ewe	N observations	n	% of obs	n	% of obs	n	% of obs
1	72	55	76.39	4	5.56	0	0.00
2	250	192	76.80	14	5.60	0	0.00
3	166	117	70.48	12	7.23	2	1.20
4	99	67	67.68	7	7.07	0	0.00
5	28	18	64.29	4	14.29	1	3.57
8	16	12	75.00	0	0.00	0	0.00
10	38	27	71.05	4	10.53	0	0.00
all ages	669	488	72.94	45	6.73	3	0.45
Week of lactation	N	n	% of obs	n	% of obs	n	% of obs
0	67	53	79.10	1	1.49	0	0.00
1	84	60	71.43	1	1.19	0	0.00
2	92	67	72.83	0	0.00	1	1.09
3	93	70	75.27	1	1.08	0	0.00
4	91	73	80.22	5	5.49	0	0.00
5	96	73	76.04	11	11.46	0	0.00
6	62	51	82.26	16	25.81	0	0.00
7	41	18	43.90	6	14.63	1	2.44
8	43	23	53.49	4	9.30	1	2.33
all weeks	669	488	72.94	45	6.73	3	0.45

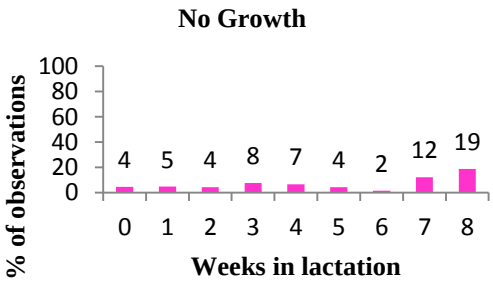
Figure A.2.4 Bacteria isolations by weeks in lactation

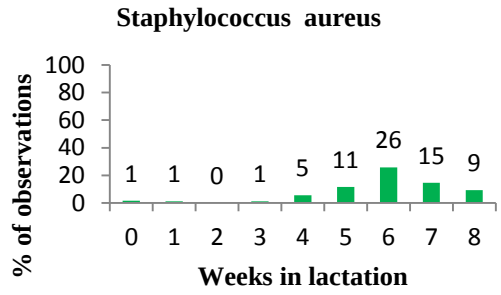



Proteus
% of observations
100
80
60
40
20
0
3 2 0 0 1 0 0 0 0
0 1 2 3 4 5 6 7 8
Weeks in lactation


Streptococcus
% of observations
100
50
0
0 0 1 0 0 0 0 2 2
0 1 2 3 4 5 6 7 8
Weeks in lactation


Coagulase negative Staphylococcus
% of observations
100
50
0
79 71 73 75 80 76 82 44 53
0 1 2 3 4 5 6 7 8
Weeks in lactation


No Growth
% of observations
100
80
60
40
20
0
4 5 4 8 7 4 2 12 19
0 1 2 3 4 5 6 7 8
Weeks in lactation


Staphylococcus aureus
% of observations
100
80
60
40
20
0
1 1 0 1 5 11 26 15 9
0 1 2 3 4 5 6 7 8
Weeks in lactation

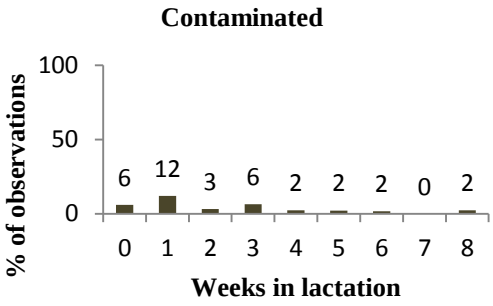
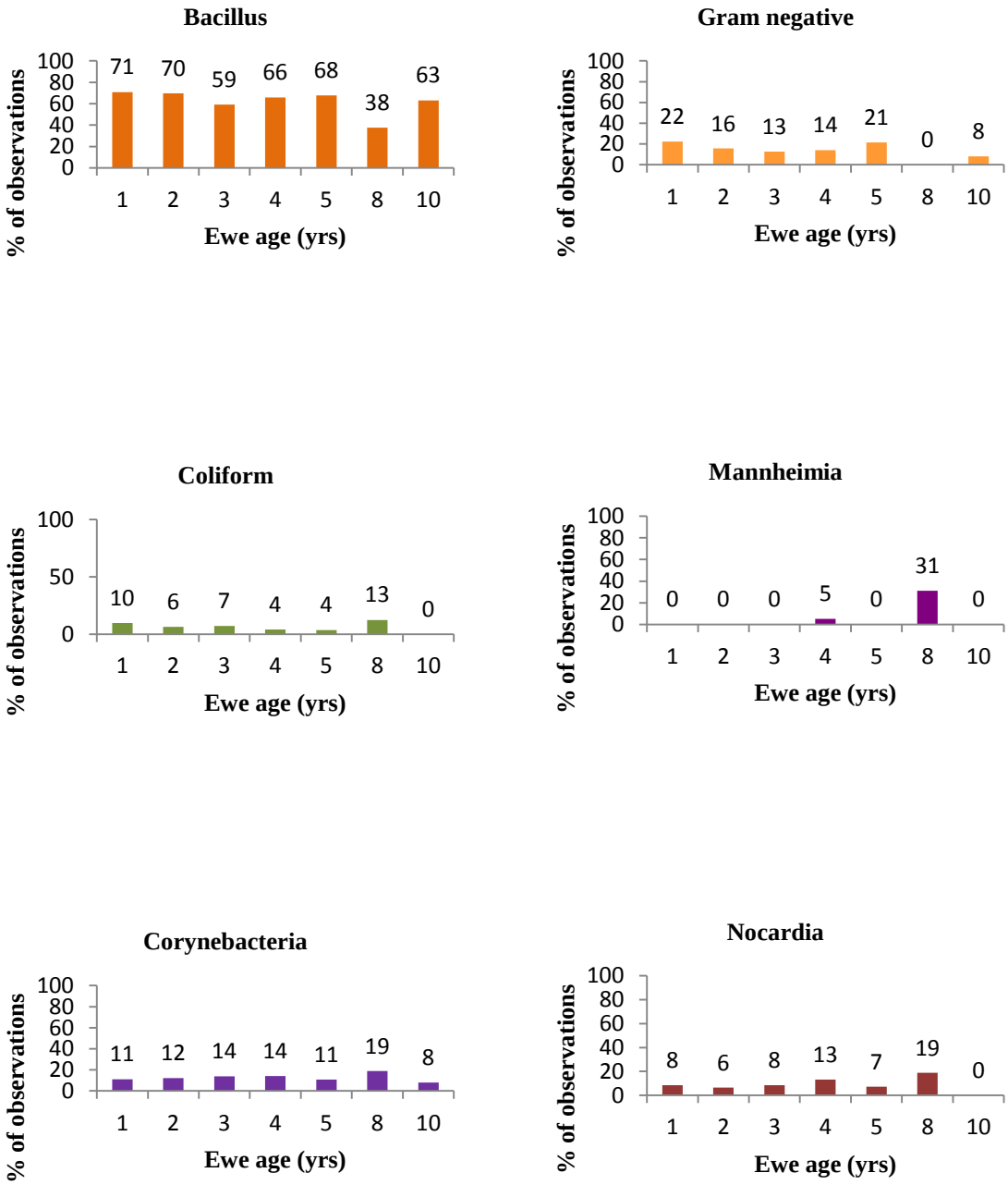
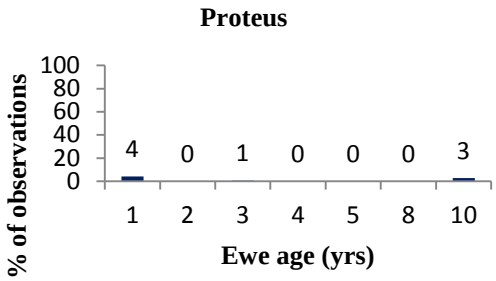
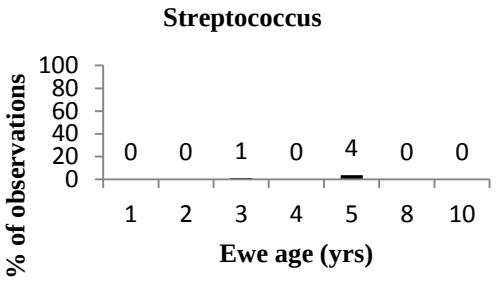
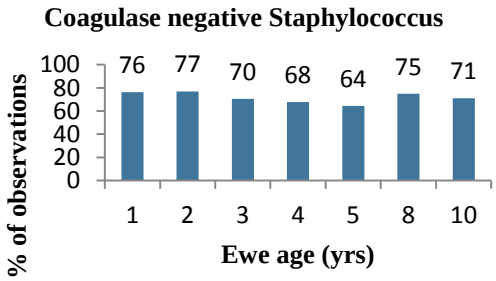

Contaminated
% of observations
100
50
0
6 12 3 6 2 2 2 0 2
0 1 2 3 4 5 6 7 8
Weeks in lactation

Figure A.2.5 Bacteria isolations by ewe age

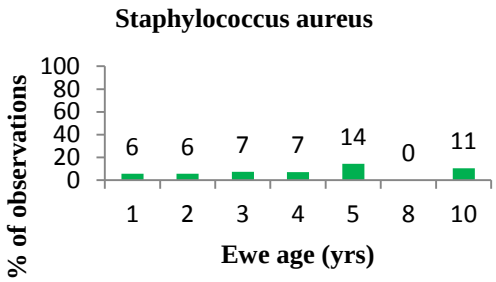



Proteus
% of observations
100
80
60
40
20
0
4 0 1 0 0 0 3
1 2 3 4 5 8 10
Ewe age (yrs)


Streptococcus
% of observations
100
80
60
40
20
0
0 0 1 0 4 0 0
1 2 3 4 5 8 10
Ewe age (yrs)


Coagulase negative Staphylococcus
% of observations
100
80
60
40
20
0
76 77 70 68 64 75 71
1 2 3 4 5 8 10
Ewe age (yrs)


No Growth
% of observations
100
80
60
40
20
0
4 4 10 7 7 6 5
1 2 3 4 5 8 10
Ewe age (yrs)


Staphylococcus aureus
% of observations
100
80
60
40
20
0
6 6 7 7 14 0 11
1 2 3 4 5 8 10
Ewe age (yrs)

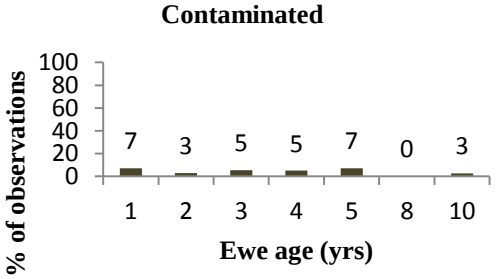

Contaminated
% of observations
100
80
60
40
20
0
7 3 5 5 7 0 3
1 2 3 4 5 8 10
Ewe age (yrs)

Figure A.2.6 Residuals plot for log_{10} HSCC with days in lactation and ewe age model

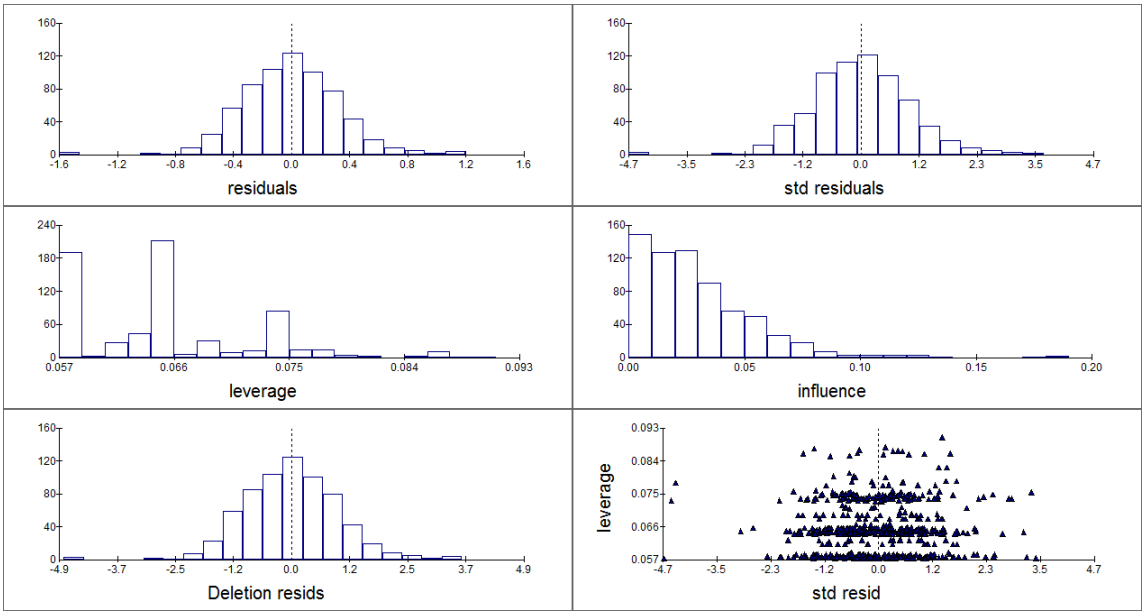
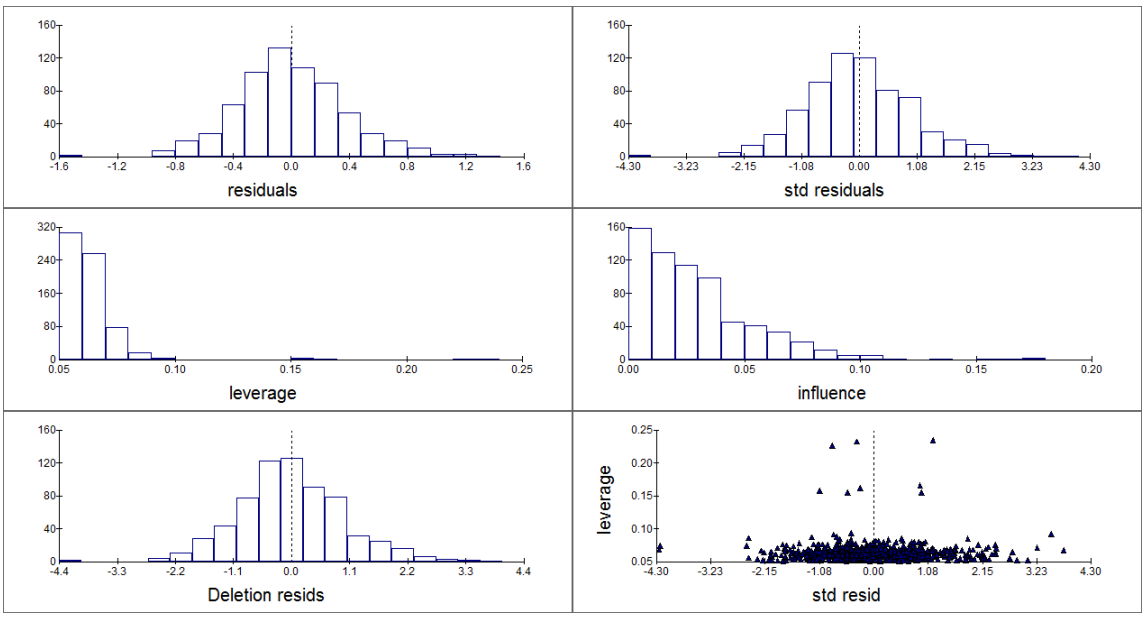


Figure A.2.7 Residuals plot for log_{10} HSCC with bacteria model



7.2 Chapter 3 Appendices

Figure A.3.1 Residuals plot for lamb weight model

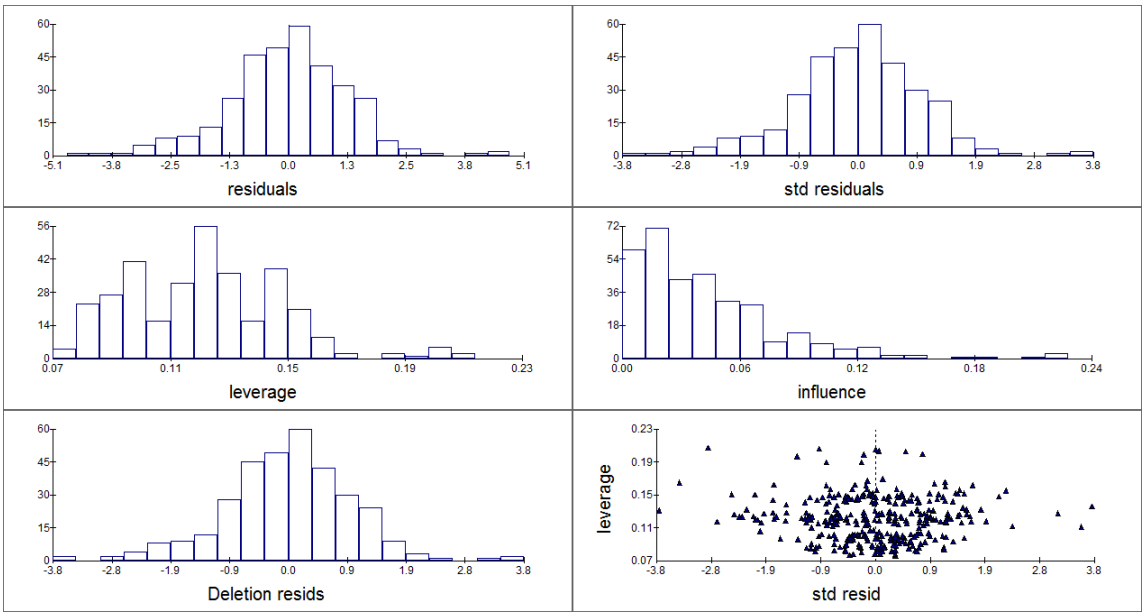
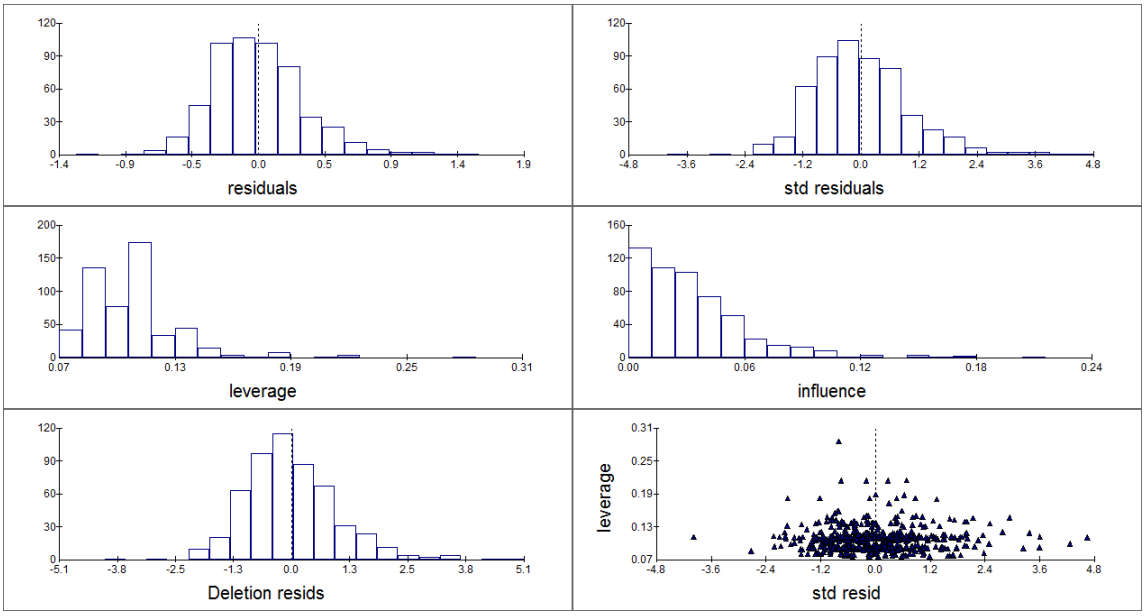


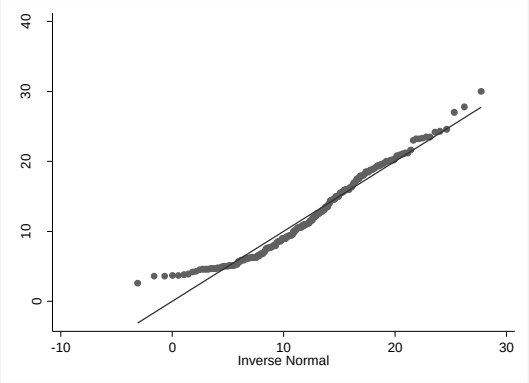
Figure A.3.2 Residuals plot for $\log_{10}$ HSCC model



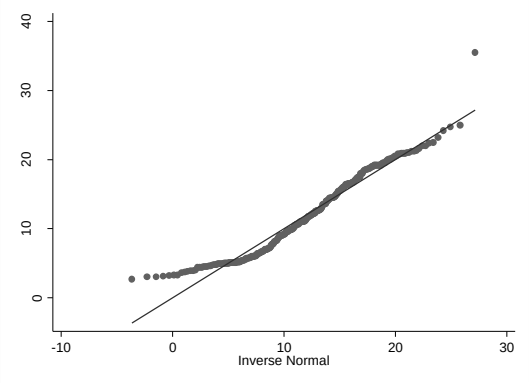
7.3 Chapter 4 Appendices

Figure A.4.1 Normal quartile plot for lamb weight for untreated and treated 3 and 6 year old ewes

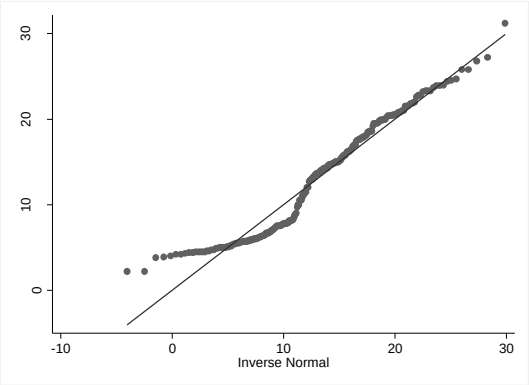
3 Year old untreated



3 year old treated



6 year old untreated



6 year old treated

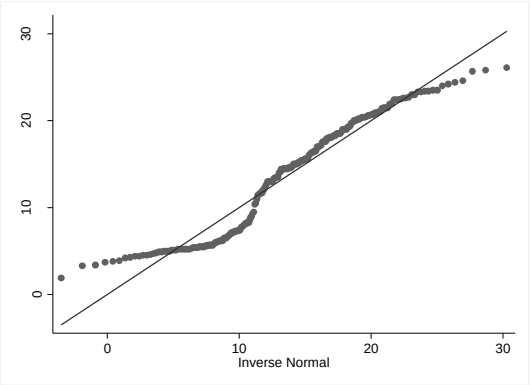
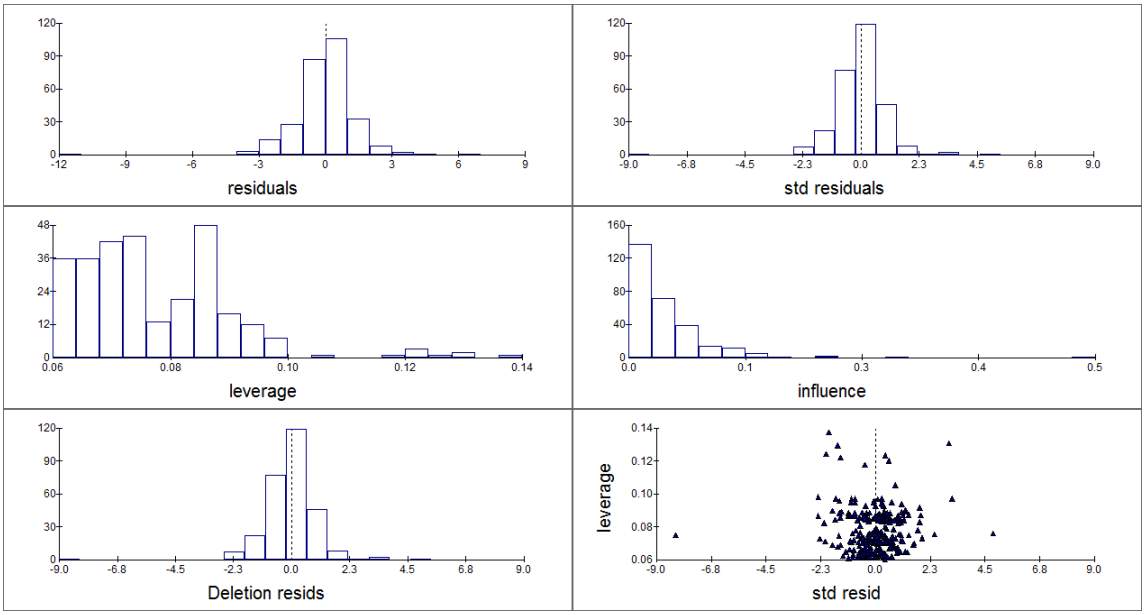


Figure A.4.2 Residuals plot of lamb weight including teat lesions model



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