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SOMATIC CELL COUNT CONTROL STRATEGIES IN DAIRY EWES

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ABSTRACT

SOMATIC CELL COUNT CONTROL STRATEGIES IN DAIRY EWES

The consumption of milk products, especially made from raw milk, have been reported to be associated with food borne diseases. Since most sheep's milk products are made from raw milk, it is clear how udder health is an important prerequisite to produce hygienic milk. Ewes with mastitis, particularly in their subclinical form, serve as reservoir of pathogens that can be shed into the milk and constitute a potential risk for human health. Milk somatic cell count (SCC) is not a public health concern itself but it is an indicator of the general state of udder health in a dairy sheep flock and can be used as an indication of hygienic milk and to improve safety of dairy products. This thesis presents three different strategies, within a comprehensive SCC control program in dairy ewes. All the studies were carried out at the University of Wisconsin, Madison dairy sheep research facility, which is the only University dairy sheep research station in North America. The study presented in Chapter 2 describes an automated method to assess SCC on farm. In Chapter 3 is presented a study aimed to determine the effect of intramammary antibiotic dry treatment on intramammary infection and somatic cell count in the subsequent lactation. Chapter 4 presents a study carried out to assess the impact of premilking teat sanitation on somatic cell count in dairy ewes. In Chapter 5 are presented the results of the combined effect of dry treatment and teat sanitation on SCC in dairy ewes.

Chapter 1
Introduction

1.0. Mastitis in dairy ewes

1.1. Definitions and classification of mastitis

The term mastitis, from the Greek words *mastos* (“breast”) and *itis* (“inflammation of”), refers to an inflammation of the mammary gland, regardless of the causative agent. Although inflammation of the mammary gland may results from trauma, injury or chemical irritation to the udder, in most of the cases mastitis derives from infection caused by microorganisms. These microorganisms, namely, bacteria, penetrate the mammary gland through the teat canal.

An intramammary infection (IMI) occurs once mastitis-causing organisms invaded the teat canal, multiply in the mammary gland, and release toxins that destroy the milk-producing tissues. The inflammation is a response of the udder intended to neutralize the infectious agents and their toxins and assist during the repair of damaged tissue and re-establish the gland to normal function. The term mastitis, unless qualified, implies the presence of an infectious microorganism.

Mastitis may occur with variety of clinical signs such as swelling, heat, redness, pain, loss of function, fever and, in the most severe cases, death. Mastitis can be characterized in various types based on the ability to detect changes in the udder or abnormalities in the milk. These signs of inflammation can be detected by visual observation or palpation. According to the degree of inflammation mastitis can be classified in:

- Clinical mastitis
- Subclinical mastitis

Clinical mastitis: occurs when the immune-system responds in such a way that visible abnormalities are present in the udder and/or milk. By means of visual observation and

palpation redness, heat, swelling, hardening and sensitivity can be detected at the udder level. Changes in milk range from the presence of flakes, clots, to watery or bloody secretions. The milk production can be negatively affected due to secretory tissue damage and the affected udder may become agalactic. In severe cases systemic response is observed with fever anorexia, depression, shock. In some cases mastitis can lead the animal to death. From a clinical point of view, mastitis takes various forms according to the level of severity:

- Subacute clinical mastitis;
- Acute clinical mastitis;
- Peracute clinical mastitis;
- Chronic mastitis.

Subacute clinical mastitis: is a mild form of clinical mastitis and the signs are mainly local. The affected udder is characterized by macroscopic or quantitative alteration of milk such as: flakes, clots, watery secretions. At the udder level slight or no heat, swelling and pain are observed. A decrease in milk production may result, but no systemic signs of disease can be detected.

Acute clinical mastitis: these cases are characterized by the rapid onset of signs at the udder level including redness, swelling, oedema, hardness, heat, asymmetry, sclerosis, pain and reduced milk yield. Milk is abnormal (serum-like, purulent or bloody secretion) and yield is usually depressed. Systemic symptoms may also be present: fever, loss of appetite, weakness, reduced rumen function, rapid pulse, dehydration and depression.

Peracute clinical mastitis: this form of clinical mastitis is characterized by the same symptoms of the acute clinical mastitis but by a very rapid onset and a greater severity.

Additional signs are shock, udder fibrosis, septicaemia, loss of muscle coordination, reduced papillary reflex. This form of mastitis can lead to coma and death.

Chronic mastitis: if the infection, with or without clinical signs, is of long duration the mastitis is described as chronic. Chronic mastitis progressively develops scar tissue, detectable by udder palpation, changes in udder conformation and reduction in milk production.

Subclinical mastitis: this is the most prevalent type of mastitis and is characterized by no detectable changes in the udder and no visual abnormalities in milk. For this reason is also referred to as "hidden" mastitis. The presence of pathogenic microorganisms in the milk can be detected only by microbiological culture, and the inflammatory response that can be detected by screening test or laboratory procedures aimed to find an increase in somatic cell count (SCC). Because of its "hidden" nature it is difficult to detect by visual observations of the udder and of the milk by herdsman and milkers. It can lead to important economic losses (decreased milk production, reduced milk quality and quality premium), and it may also be difficult to treat with antibiotics. Subclinical mastitis constitutes a reservoir of infection to other animals. Subclinical mastitis can be diagnosed by microbiological culture of milk samples, or detected by indirect test, such as California Mastitis Test (CMT) or other test aimed to evaluate the SCC in milk. Subclinical IMI represent reservoirs of infection and the transmission mainly occurs during milking.

Mastitis can be separated into two types according to the source of the microbes causing the mastitis:

- Contagious mastitis;
- Environmental mastitis;

Contagious mastitis: is an infection of the udder with microbes that originated in the udder of another infected animal. With only a few exceptions, the microbes that cause contagious mastitis enter the uninfected udder through the teat canal. The infection is usually spread during milking. The microbes that cause contagious mastitis are adapted to live in the udder and, as a result, they can survive for long periods of time in an infected udder. In some cases, the infection may last for the entire life of the animal.

Environmental mastitis: results from infection of the udder by microbes that come from the environment. Sources of microbes that cause environmental mastitis include: manure, bedding, feed, dust and dirt, mud, water and contaminated equipment.

Contagious and environmental mastitis superimpose with the classification based on the clinical signs so that we can distinguish clinical and subclinical mastitis due to contagious and environmental microorganism.

1.2. Udder defence mechanisms and development of mastitis

Mastitis develops as a result of the interaction of a pathogen microorganism and the mammary gland. The udder has three order of defense mechanisms against the bacteria, the first is mechanical or anatomical and it is represented by the teat canal, the second is an immunological defense (cellular and humoral immune-system), and the third is a set of non specific immune-factors.

Teat canal

The teat canal represents a physical barrier to the penetration of bacteria. The sphincter muscle surrounding the teat duct maintains the teat orifice close and avoids

bacterial penetration. The teat canal is occluded by the keratin, a waxy substance, derived from the teat duct lining. Keratin contains antibacterial substances (basic proteins and fatty acids). The colonization of bacteria is also reduced by epithelial desquamation and by the flushing action of milk during milking.

Immunological defense mechanism

The immune-system of the mammary gland consists of:

- cellular component (polymorphonuclear neutrophilic leukocytes, lymphocytes and macrophages) ;
- humoral component (immunoglobulins);

The cellular component includes the white blood cells (leukocytes), normally present in a healthy udder and others that are activated by the immune-system of the mammary gland. Among the different types of cells involved in the cellular immune system the most important are lymphocytes and macrophages. Milk leukocyte are normally present in the milk of an uninfected mammary gland, but their number increase in response to invading pathogens and can reach concentration of million/ml. During inflammation the predominant type of leukocytes are the polymorphonuclear neutrophilic leukocytes (PMN) which enter the mammary gland from the blood. The function of PMN is to engulf the microorganisms and kill them (phagocytosis). Other types of leukocytes are the lymphocytes and macrophages. The lymphocytes coordinate the immune-system response by the release of mediators called cytokines. Cytokines are hormone-like proteins that stimulate the recruitment of cells into the infected udder and promote the production of antibody-producing plasma cells from activated lymphocytes. The macrophages play a role in the phagocytosis and destruction of bacteria.

They have a major role in the modulation of the immune response releasing cytokines and leukotriens with the function of recruit lymphocytes.

The humoral immune system consists of immunoglobulins which contain specific antibody activity against alien antigens. Milk antibodies are specific immune factors that help in controlling bacterial infection. In normal condition their concentration in milk is low, but they increase during inflammation of the mammary gland. The concentration of antibodies is high in colostrum. Their action is to coat bacteria (opsonization) to facilitate the phagocytosis by PMNL and macrophages. Antibodies are capable to neutralize toxins and in some case they have a direct bactericidal action. An additional function of antibodies is to interfere with the adhesion mechanism of bacteria to the epithelial surfaces.

Non specific immune factors

Other antimicrobial factors are present in milk such as enzyme systems (lactoperoxidase and lysozyme) and proteins (lactoferrin, transferring and complement).

Lactoferrin and transferrin are the most important iron-binding protein in secretions (such as milk) and in the circulating body fluids, respectively. These proteins limit the growth of bacteria by subtracting iron necessary for bacterial metabolism. Their content increases markedly during intramammary infections. Lysozime is an enzyme that hydrolyses β-bonds of the bacterial wall and as a consequence of the osmotic lysis has a direct bactericidal effect. The antibacterial activity of the lactoperoxidase system is based on the oxidation of sensitive enzyme structures within the bacterial wall. The complement function is to recognize and destroy microbes and direct the phagocytes to their target (opsonization, chemotaxis).

An intramammary infection begins when microorganisms penetrate through the teat canal and then multiply in the mammary gland. In order to develop mastitis, the following steps are necessary:

- Invasion of the mammary gland;
- Establishment of infection;
- Destruction of alveolar tissue;
- Mammary inflammation.

Invasion of the mammary gland

The teat represents a mechanical defense against the penetration of bacteria into the udder. In normal condition the teat canal is tightly closed by the sphincter muscle in the interval between milkings. Microorganisms can penetrate the teat during machine milking. Organisms present in the milk or at the teat end impact the teat end orifice by means of vacuum fluctuations. Liner slipping and removal of teatcups without first shutting off the vacuum are common causes of vacuum fluctuation. In the case of hand milking, milker's hands can also represent a vector for bacteria. Transmission may occur also by suckling lambs that can spread microorganisms from infected to healthy udders. Microorganisms can penetrate the teat canal also by multiplying inside the canal, or they can be pushed through contaminated cannulae during intramammary antibiotic treatment.

Establishment of infection

Once the bacteria enter the mammary gland their chances to induce an infection depend on the ability to stick to the udder tissue and remain into the affected gland. Bacteria first attack the tissues lining the large milk-collecting ducts. Pathogen microorganisms developed different strategies to colonize the mammary gland. Contagious bacteria such as *Staphylococcus aureus*

and *Streptococcus agalctiae* are able to adhere to the tissue lining the teat and gland cistern. Other, such as *Escherichia coli*, multiplies rapidly inside the mammary gland. The establishment of an infection is contrasted by leukocytes (white blood cells) naturally present in the milk. These cells represent the second line defense because they can engulf (this process is called phagocytosis) and destroy bacteria. During this process, the leukocytes release substances (linfokines) that cause the movement of additional leukocytes from the blood into the milk. If the infecting microorganisms are eliminated, the infection is cleared, but if bacteria are not entirely destroyed, they continue to multiply and begin to invade smaller ducts and alveolar areas.

Destruction of alveolar tissue

Bacteria produce toxins that damage milk-secreting cells. These damaged cells release substance that attract additional leukocytes move to the site of infection. Leukocytes enter the alveolar tissue in great numbers by squeezing between the loosen junction of milk secreting cells. Damaged cells also release irritant substances that increase the permeability of blood vessels, leading to leak of fluids, minerals and clotting factors in the attempt of dilute bacterial toxins. In some cases the damaged alveolar structures are replaced by connective and scar tissues, and this is the cause the of reduced milk production.

Mammary inflammation

Leukocytes and fluids that flow from the blood are responsible for the local swelling of the udder. In some cases the mammary inflammation may be mild and go undetected (subclinical mastitis), or may produce a severe response with visible signs (clinical mastitis). This signs are characterized by redness, swelling, edema accompanied by abnormal secretion of milk (watery, presence of clots, flakes and red blood cells in the most severe cases).

Susceptibility of mastitis

The incidence of clinical IMI does not vary with the lactation stage in the same way as in dairy cattle. A high incidence at drying-off or at parturition is observed in very rare and specific cases (mycotic agents or *P. aeruginosa*). In small ruminants the higher rates are observed at the beginning of machine milking and during the first third of lactation. High incidence may be observed in dairy ewes during suckling-milking periods (Bergonier et al., 2003).This higher incidence during these periods is associated with climatic conditions such as cold temperature and wet weather during winter season (Burmeister et al., 1995; Burriel, 1997b), nutrition factors (lush pastures rich with high protein contents and low energy intake) and transition stress between hand and machine milking.

1.3. Epidemiology of mastitis in dairy ewes

There is a large literature on mastitis, relative to dairy cattle, but much less information is available for dairy sheep. Most of the research has been carried out in Mediterranean countries, where the dairy sheep has a long tradition. The reports are inevitably different according to the different breeds, rearing system, environment and experimental designs. The annual incidence of clinical mastitis in small dairy ruminants is estimated to be less than 5%, whereas the prevalence of subclinical mastitis ranges between 5-30% or higher in some cases (Lafi et al., 1998; Las Heras et al., 1999; Albenzio et al., 2002; Bergonier and Berthelot, 2003; Contreras et al., 2003). Little is known on the incidence of IMI in dairy ewes. *S. aureus* has been reported as the most recovered pathogen from dairy ewes with clinical mastitis (Al-Samarrae et al., 1985; Kirk et al., 1996; Lafi et al., 1998; Ariznabarreta et al., 2002;). Although other pathogens such as *Streptococcus spp*., *Enterobacteriaceae*, *Pseudomonas aeruginosa*,

Mannheimia haemolytica, *Corynebacteria* and fungi can cause clinical mastitis in small ruminants, their occurrence is lower (Quinlivan, 1968; Jones, 1991; Lafi et al., 1998; Leitner et al., 2001; Bergonier and Berthelot, 2003). The literature reports cases of mastitis following intramammary infusion of antibiotics and attributed to *Aspergillus fumigatus*, *Serratia marcescens*, *Pseudomonas aeruginosa* (Berthelot and Bergonier, 1993; Jensen et al., 1996; Pérez et al., 1998; Las Heras et al., 1999, 2000). *Mycoplasma spp* IMI are undervalued because the animals are usually culled for symptoms other than mastitis. Mycoplasmoses are diseases affecting small ruminants around the world, and endemically in the Mediterranean Basin (contagious agalactia). *Mycoplasma agalactiae* outbreaks in small ruminants are responsible for large increases in bulk tank milk somatic cell count (BTSCC) and reduction in milk production, reasons to consider contagious agalactia one of the most important causes of mastitis in endemic areas (Corrales et al., 2004). Coagulase Negative Staphylococci (CNS), considered as minor pathogens when isolated in dairy cows (Schalm et al., 1971), have been frequently reported to be the most commonly isolated pathogens recovered from cases of subclinical mastitis of dairy ewes and they account up to 93% of the cases (Fthenakis, 1994; Gonzalez-Rodriguez et al., 1995; Burriel, 1997; Lafi et al., 1998; Las Heras et al., 1999; Pengov, 2001; Leitner et al., 2001; Ariznabarreta et al., 2002; Gonzalo et al., 2002; Hariharan et al., 2004). Furthermore, CNS in dairy ewes elicit SCC levels higher than $1.0\text{-}1.5x10^6$ (Pengov, 2001) and may even cause clinical mastitis (Fthenakis and Jones, 1990b) in a fashion similar to major pathogens. For these reasons CNS in dairy ewes cannot be considered minor pathogens. The most commonly isolated CNS species in subclinical IMI in ewes are *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus chromogenes* and *Staphylococcus xylosus* (Burriel, 1998; Ariznabarreta et al., 2002; Gonzalo et al., 2002; Bergonier et al., 2003).

Staphylococcal subclinical IMIs in dairy ewes tend to be persistent and develop frequently into chronic mastitis. They can persist up to 3 months or more (Bergonier et al., 1996). Variations in the prevalence of subclinical IMI are reported on the basis of the lactation stage, with high incidence at the beginning of the lactation (Kirk et al., 1996; Leitner et al., 2001). IMIs prevalence tend to increase as the lactation proceed for some authors (Watson et al., 1990; Watkins et al., 1991; Fthenakis, 1994) while it declines with time post partum for other authors (Hueston et al., 1986; Kirk et al., 1996; McDougall et al., 2002). The prevalence of IMI has been reported to be higher as the number of lactation increases (Watson et al., 1990; Watkins et al., 1991; Fthenakis, 1994; Sevi et al., 2000; Leitner et al., 2001). A review of five studies, by Bergonier and others (2003) showed that the majority of cases of mastitis occurred from the beginning of machine milking and during the first third of lactation. Other studies have reported mastitis occurring from the first week postpartum (Onnash et al., 2003) to three weeks after drying off (Saratsis et al., 1998); according to Bergonier et al. (2003) mastitis at drying off is unusual, and caused mainly by different pathogens related to poor environmental hygiene. Caution should be used in interpreting the prevalence of subclinical IMI, since different criteria have been used to define subclinical mastitis.

1.3.1. *Microorganism that cause mastitis*

Mastitis is the result of the interaction between the udder, the environment and the microorganisms. Although a wide variety of microorganisms can cause mastitis, including bacteria, mycoplamas, yeast, algae, fungi, and on rare occasion viruses, most cases of mastitis are caused by bacteria. The most frequently isolated microorganisms in small ruminants IMI are *Staphylococcus* spp. Other pathogens such as *Streptococcus* spp, *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Manheimia haemolitica* and *Corynebacteria* can cause IMI but with

a lower occurrence (Quinlivan 1968; Kirk et al., 1980; Hueston et al., 1986, 1989; Fthenakis, 1994; Lafi et al., 1998; Al-Majali and Jawabreh, 2003). The pathogenic microorganisms responsible for mastitis can be divided in four categories based on their source: contagious pathogens, environmental pathogens, opportunistic and miscellaneous infections.

The distinction is important on a practical basis, because the strategies to control mastitis differ on the basis of the microorganism involved.

Contagious pathogens. The microbes that cause contagious mastitis are adapted to live in the udder and, in most of the cases, they enter the uninfected udder through the teat canal. An exception could be mycoplasmal infections, these may originate in other body sites and spread systemically to the udder. In some cases contagious pathogens can establish subclinical infections and survive for long periods of time in the infected udder (chronic infections). They are shed in milk of the infected udder which is the main source of these bacteria. They are spread from animal to animal during the milking by means of milking machine equipment (clusters), milker's hands and, when used, udders wash cloths. Common contagious pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma bovis*.

Staphylococcus aureus is the most common isolate from cases of clinical sheep mastitis (Quinlivan, 1968; Watson and Buswell, 1984; Al-Samarrae et al., 1985; Lafi et al., 1998) and the infected udder is the most important source of the pathogen that shed the pathogen in milk (Schalm and Kendrick, 1956). It can be associated with acute gangrenous mastitis (bloody milk, udder necrosis). The organism, once into the mammary gland, colonizes the alveoli producing damage to the milk-producing tissue, which is replaced by fibrotic tissue. As a consequence there is a consistent decrease in milk production. *S. aureus* can establish pocket of infection with abscess formation often followed by walling-off of bacteria by scar tissue. Shedding of

bacteria in milk is occasional so that high bacteria counts in bulk tank milk are generally not observed, although the bulk tank SCC may be high. Chronic mastitis with non-encapsulated active lesions may be associated with intermittent shedding of bacteria and elicit high SCC. This phenomenon is responsible for the low cure rate with antibiotic therapy. An additional protection of *S. aureus* against antibiotic treatment is supplied by the production of exopolysaccarides that surround the bacteria (Baselga et al., 1994). Control strategies of gangrenous mastitis include vaccination and culling. However, the vaccination against *S. aureus* gangrenous mastitis, proved to control only clinical cases (Marco, 1994). Ewes chronically infected with *S. aureus* should be culled.

Streptococcus agalactiae although it can live outside the udder for short periods of time, it is considered to be an obligate pathogen of mammary gland. It is a common mastitis agent whose in most of the cases shows no or few clinical signs of mastitis, such as abnormal milk. Streptococcal mastitis is usually associated with high somatic cell counts and decrease in milk production. Mastitis caused by *Streptococcus agalactiae* should be suspected when bulk tank SCC rise and remain high, with no signs of clinical infection. The cisterns and the ductal system are the elective sites of streptococcal colonization. Irritants and bacterial waste products are responsible of the inflammation and can result in destruction of milking producing tissue, with subsequent reduction in milk yield, and in the most severe cases, agalactia. Occasional shed of *Streptococcus agalactiae* from the infected udder can be detected by high bacteria counts in bulk tank milk.

*Mycoplasma*s are not classified as viruses nor bacteria, but as intermediate organisms somewhere in between. They are highly contagious organisms. These microorganisms lack of a cell wall, which explains why mycoplasmal infections do not respond to antibiotic therapy. *Mycoplasma* spp. is characterized by a high morbidity and large number of microorganism can

be shed in the milk. Mycoplasmal infections should be suspected in flocks with marked drop in milk production, high somatic cell count and with little evidence of clinical mastitis. Especially in cases unresponsive to treatment and in flocks where new animals have been introduced.

Environmental pathogens. These types of bacteria arise in the environment, particularly in udder exposed to faeces, soil, mud, dirty bedding materials. Although passage from an infected to uninfected udder can occur, they cannot be eliminated from the environment, so this represents the major source of the infection. The environmental bacteria that cause mastitis are environmental streptococci (other than *Streptococcus agalactiae*), enterobacteria, enterococci and *Pseudomonas* spp. Enterobacteria and enterococci mastitis seems to be less common among small ruminant when compared with cattle and their primary source is the litter. Commonly isolated coliforms from sheep include *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* as the most common, with *Salmonella spp*. being more rarely isolated. *Pseudomonas aeruginosa* has been implicated in outbreaks of acute/peracute mastitis with high levels of mortality in lactating dairy sheep. Pseudomonas is found especially in water or humid environment and it could persist in the pipeline and teatcups of the milking equipment due to aptitude to produce biofilm. Coliform mastitis is more common in the post-parturient period and is associated with severe systemic disease. It can be either a persistent or transient infection. *Environmental Streptococci*: streptococci are probably the second group of microorganisms in importance, after staphylococci, responsible for mastitis in sheep (Bergonier et al., 1999). Their primary sources are infected animals, litter, and the environment. *Streptococcus uberis* is frequently recovered in farms where some failure in milking procedure occurred (e.g. poor milking machine maintenance or settings).

Opportunistic microorganism. Staphylococci are a group of microorganisms that normally live on the teat skin of dairy ewes (Burriel, 1997; Scott and Murphy, 1997). They represent the

most frequent isolates from subclinical mastitis (Hueston et al., 1986, 1989; Kirk et al., 1980, 1996; Fthenakis, 1994; Las Heras et al., 1999; Al-Majali and Jawabreh, 2003), but they're capable of producing mastitis in clinical form (Watson and Buswell, 1984). They cannot be classified as contagious or environmental pathogens, but are referred as opportunistic. They include a group of *Staphylococcus* species other than *Staphylococcus aureus* (Coagulase Negative Staphylococci or CNS). Although CNS live on teat skin, accessory sources are housing, bedding, feedstuff, air, insects, clusters, equipments and humans (hands). The main factors of transmission are milking machines, especially liners, and sometimes milker's hands. Risks of transmission occur during milking when milkers have a poor routine with overmilking, when they strip ewes at the end of milking or remove clusters without shutting off the vacuum; then, likelihood of impacts of infected milk droplets against teats is very high.

Mycotic mastitis. Fungi (moulds and yeast) are common environmental organisms (Kirk and Bartlett, 1986) and they can be found in different substrates such as soil (Richard et al. 1980), plants, bedding material (Loftsgard and Lindquist, 1960), feed and water (Elad et al., 1995; Hintikka, 1995). They're normally present on the skin of the udder and teats in low numbers (Loftsgard and Lindquist, 1960; Farnsworth and Sorensen, 1972) and act as opportunist pathogens of the mammary gland and cause mastitis when udder natural defence is lowered. Mycotic mastitis is a sporadic condition in dairy cattle, with an incidence of 1-12% of all clinical cases (Van Damme, 1983; Costa et al., 1993; Aalbaæk et al., 1994; Krukowski et al., 2000;). The incidence of mycotic mastitis is usually associated with intramammary infusion of antibiotics when contaminated syringes are used (Paine, 1952; Loftsgard and Lindquist, 1960; Mantovani et al., 1970; Farnsworth and Sorensen, 1972; Thompson et al., 1978; Richard et al., 1980; Kirk and Bartlett, 1986; Krukowski et al., 2000;). Yeast are normally present on the skin of the udder and teats (Richard et al., 1980) and may enter the teat canal either by means of

inappropriate use of instruments, such as cannulae or syringes, or contaminated antibiotic preparations used for infusion (Sheena and Siegler, 1995). Once yeast penetrates into the mammary gland, their growth might be promoted by the antibiotic inhibiting bacterial population (Farnswoth, 1987; Thompson et al., 1978; Berteloth and Bergonier, 1993; Jensen et al., 1996; Pérez et al., 1998). Thus, during bacterial infection yeast may multiply in necrotic tissue and the yeast infection follows a primary bacterial infection. Yeast infection could be suspected in mild cases that don't respond to antibiotic treatment. Little is known about mycotic mastitis in sheep and goats (Jensen et al., 1996). Few cases of mycotic mastitis by *Aspergillus fumigatus* have been reported in small ruminants (Berthelot and Bergonier, 1993; Jensen et al., 1996; Pérez et.al, 1998, 1999). An association between mammary aspergillosis and incorrect administration of antibiotic at drying-off was suggested (Las Heras et al., 2000). Antibiotic dry therapy should be performed under proper hygienic conditions, using sterile products and equipment and following proper sanitary procedures.

M*iscellaneous Infections*. *Serratia marcescens* has been associated with contaminated teatcups and is also a common environmental pathogen in cattle. *Pasteurella hemolytica* is normally recovered from the upper respiratory tract of sheep and lungs of pneumotic sheep. It is commonly associated with case of peracute mastitis (Shoop and Myers, 1984; Watson and Buswell, 1984; Hueston et al., 1986) often referred as "blue bag" mastitis. The source of infection is from the nose and throats of nursing lambs and the transmission is by means of suckling lambs, but also insects or flies. Cold and wet conditions improve the survival of *P. hemolytica.* On rare occasions *Bacillus* spp. (Watson and Buswell, 1984) and *Actinomyces* (*Corynebacterium*) *pyogenes* (Kirk et al., 1980; Watson and Buswell, 1984; Fthenakis, 1994) can be recovered from infected glands.

1.3.2. Somatic Cell Count

Somatic cell (literally "body cells") is a term that refers to cells that are naturally present in the milk and their count, somatic cell count (SCC), is expressed as number of cells "per millilitre"(cells/ml). The total SCC of milk is made up of two types of cell:

- mammary gland cells: these cells (epithelial cells and eosinophils), are part of the normal turnover of the mammary gland and they are shed in milk as they are renewed;
- white blood cells (macrophages, lymphocytes and neutrophils or polymorphonuclear neutrofilic leukocytes), derive from the blood and serve as a defence mechanism, and in repairing damaged tissue in the mammary gland.

The somatic cells types in milk from ewes free of IMI are very similar to those observed for cows (De la Cruz et al., 1994; Gonzalez-Rodriguez et al., 1995). In sheep milk samples collected from uninfected gland 2-3 % of the overall SCC are epithelial cells, 10-35% polymorphonuclear neutrophil leukocytes (PMNL), 45-85% are macrophages and 11-20% lymphocytes (Paape et al., 2001; Bergonier et al., 2003). Thus, white blood cells represent the most prevalent cell type in milk.

SCC levels tend to be higher in sheep's milk than in cow's milk (Green, 1984; Maisi et al., 1987: Gonzalo et al., 1994b; González-Rodríguez et al., 1995), but with a dynamic that is similar for both species (Cuccuru et al., 1997). In dairy cows SCC in milk from uninfected quarters is usually < 100,000 cells/ml (Hillerton, 1999). Many research tried to define the SCC level of a healthy udder in dairy ewes, reporting values similar to those from cows, <100-300 × 10^3 cells/ml (Zarzycki et al., 1983; Fruganti et al., 1985; De la Cruz et al., 1994; Gonzalez-Rodriguez et al., 1995; Romeo et al., 1996; Paape et al., 2001). Other authors reported values of 500 ×

10^3/ml up to 1600 × 10^3/ml (Green, 1984; Mackie and Rodgers, 1986; Maisi et al., 1987; Fthenakis et al., 1991; González-Rodríguez and Carmenes, 1996; MacDougall et al., 2001). SCC in milk samples from infected udders ranged between 1.4 × 10^6 cells/ml and 2 × 10^6 cells/ml (Fhenakis, 1994; Mavrogenis et al., 1995; Romeo et al., 1996; Burriel, 1997). This higher SCC could be due either to differences in breeds, husbandry, management measures (milking routine and dry therapy) or to a real difference between cattle and sheep.

The function of the somatic cells is to contribute to the immunoresponse during inflammatory status of the mammary gland and to assist in the repairing of damaged secretory tissue. When an infectious agent enters the udder or when the udder is injured the SCC will rise, so it is used as an indicator of the health of the udder and as an indirect method to detect intramammary infections in their subclinical form (Coffey et al., 1986; Gonzalo et al., 1993; González-Rodríguez et al., 1995; Gonzalo et al., 2002). SCC and PMNL are highly correlated as the increase of SCC in ewes is due mainly to PMNL, which can increase up to 90% of somatic cells in milk (Cuccuru et al., 1997; Moroni and Cuccuru, 2001).

SCC of culture-negative udder halves were significantly different ($P < 0.001$) from those infected (Berthelot et al., 2006).

Somatic cell count can be related to physiological and pathological variation factors, although the greater increase is induced by infection of the gland. Among the physiological factors are:

Flock management mild variation of SCC, lower than 20,000 cells/ml, can be accounted to management of the flock, i.e. number and suckling-milking period of lambs, the lambing month, and dietary (Lafi et al., 1998; Rupp et al., 2003).

Breed: literature reports wide SCC discrimination values between healthy and infected glands (Berthelot et al., 2006), suggesting that the breed effect is a significant factor affecting SCC. González-Rodríguez et al. (1995) pointed the need to use relative thresholds for each breed;

Stage of lactation: high SCC values are detected the day of lambing (596 × 10^3 cells/ml) and tend to decrease during the transition from colostrum to true milk until minimum values at the peak of lactation (e.g. 30 × 10^3/ml) at the fifth week of lactation. The counts tend to stay unchanged until the end of lactation (Paape et al., 2001; Bergonier et al., 2003). In the last month before drying-off when the sheep are milked once a day SCC mean values higher than 4×10^5/ml are recorded and even higher values (6-8×10^5/ml) could be detected in the last two weeks when ewes are milked one time every two or three days (De Santis et al., 1998).

Season

Milk yield: a moderate negative correlation (from 0.09 to 0.37) exist between milk yield and SCC level (Baro et al., 1994; Gonzalo et al., 1994; Bedö et al., 1995; Fuertes et al., 1998; El-Saied et al., 1999; Rupp et al., 2001, 2003; Othmane et al., 2002).

Diurnal variation: a variation of SCC between morning milking and evening milking has been observed and it is due to the time intercurring between milkings. Usually, higher values are reported for the p.m. milking. The p.m. milking is characterized by lower milk yield, higher fat and protein contents, thus these differences are probably related with a dilution effect in the period between milking. The dilution effect may be responsible also for the higher SCC level detected at 1 hour after milking. (Gonzalo et al., 1994a; De la Fuente et al., 1997).

Number of lambs: although the number of lambs delivered at lambing does not influence the SCC (Gonzalo et al., 1994), the lamb weaning causes a mild increase of SCC in milk during the first two weeks (Gonzalo et. al., 1985).

Parity: the lactation number of the ewes affects milk composition. The mean SCC increase between the first and fourth lactation by 4 to 11% (Gonzalo et al., 1994b; Lafi et al., 1998; Olivetti et al., 1988; Pulina 1990; Bergonier et al., 1996).

Milk fraction during milking: the fraction of milk significantly affects the content of SCC milk. Definition of milking fraction. The stripping fraction was 1.7 times higher than the corresponding measurement in the foremilk fraction. Although the machine milk SCC did not differ from that of the foremilk fraction, it was significantly less than the stripping milk (Peris et al., 1991).

Storage method and milk sample age: a study showed how refrigerated and frozen milk have lower SCC values when compared to fresh milk and how SCC values tend to decrease during storage, indicating that the more accurate estimation of the SCC are obtained with fresh milk (Gonzalo et al., 1993).

Although many non infectious factors contribute in SCC variation, IMI is the main variation factor of SCC, and represents the best predictor of infection status among the indirect tests available at the moment (Green, 1984; MacDougall et al., 2001; Bergonier and Berthelot, 2003). In order to distinguish between physiological from pathological cell variations a differential cell counts can be applied.

In ewes with subclinical IMI SCC can increase up to 1×10^6 SCC/ml without macroscopic abnormalities in milk (Green, 1984; Fthenakis et al., 1991; Gonzalo and San Primitivo, 1998).

Somatic cell counts represent therefore, the intensity of the cellular immune defence in response to an inflammatory process and can be used as an indirect test for the detection of subclinical mastitis (Green, 1984; Paape et al., 1984). The individual SCC (iSCC) of a ewe is an

indication of the health status of the mammary gland, and bulk tank milk SCC (BTSCC) can indicate the general state of udder health in a sheep flock.

The individual SCC (iSCC)

The SCC is a useful predictor of IMI in dairy ewes (González-Rodríguez et al., 1995; Barillet et al., 2001; Gonzalo et al., 2002). There is no agreement in the definition of a SCC threshold that can permit to discriminate between healthy and infected glands in dairy ewes. The simplest method to discriminate between 'healthy' and 'infected' udders is to use a single threshold and a punctual approach. Many cut-off points have been proposed by the different authors to differentiate between infected and non-infected glands or animals ranging from 200,000 to 2,000,000 cells/ml (Green, 1984; Mackie and Rodgers, 1986; Maisi et al., 1987; Fthenakis et al., 1991; González-Rodríguez et al., 1995; Mavrogenis et al., 1995; Fthenakis, 1996; Pengov, 2001; McDougall et al., 2001; Berthelot et al., 2006), suggesting differences in breeds, geographical area, management conditions and type of milking.

While a dynamic approach has been developed in dairy cows over the last twenty years (Dohoo and Leslie, 1991; Romeo et al., 1996; Suarez et al., 2002; Berthelot et al., 2006). Bergonier et al. (1996) suggested the use of a dynamical and multiple thresholds approach rather than the use of punctual approaches. Selecting two thresholds (500,000 and 1,000,000 cells/ml) they divide the population of ewes into 3 classes on the basis of individual data during the entire duration of the lactation: healthy, "doubtful" and infected. An udder is considered as uninfected, if every SCC measurement (except two) does not exceed 500,000 cells/ml during the whole lactation. An udder is infected if at least two SCC measurements during the lactation exceed 1,000,000 cells/ml. The infection status is doubtful in all other cases.

Individual somatic cell count (iSCC) are used to make management decision, such as select the ewes for culturing, dry treatment or culling, as a part of mastitis control program.

Bulk tank milk SCC

The bulk tank SCC (BTSCC) could be used as a predictor of the prevalence of mastitis in the flock (Lagriffoul et al., 1999). Where milk quality system payment are applied the interest in monitoring BTSCC has greatly increased because of the strong relationship (R^2 = 0.79) existing between the annual BTSCC and the estimated prevalence of infected ewes in a flock. An increase of 100,000 cells/ml is associated with an increase of prevalence of 2.5% (Berthelot et al., 2006). For small ruminants the BTSCC has a legal limit in the United States (US) established by the Food and Drug Administration of 1,000,000 cells/ml, whereas no legal limit has been established in the European Union (EU). Where BTSCC are monitored on a regular basis by dairy improvement agencies, this constitutes a guideline for identification of mastitis problems in the flock. On a practical basis, when BTSCC rises, producers must be aware that possible udder health problems are present, even if ewes may not be exhibiting clinical signs of mastitis. Monitoring BTSCC values is only indicative of subclinical mastitis problem, but there is no simple method to estimate the real prevalence of subclinical mastitis other than individual SCC.

1.3.3. Diagnosis of mastitis

As a consequence of the inflammation, changes occur in the tissue and in the milk. Early detection of mastitis is essential for an effective control programme. The diagnosis of mastitis is generally based upon: clinical examination (visual observation of milk and udder palpation), bacterial culture and enumeration of somatic cells.

Clinical examination. Sign of redness or swelling can be detected by a simple visual exam of the udder. Palpation of the udder and lymph nodes is a procedure that should be performed after milking, when it is easier to detect swelling, hot to the touch tissues, nodules, fibrotic tissues and pain. A complete exam includes also the observation of the foremilk using a filter cup looking for the presence of flakes, clots, watery secretion, blood, etc. Since these symptoms are often absent, particularly in cases of subclinical mastitis infections, the clinical exam is useful to detect clinical or chronic mastitis while it is not suitable in case of subclinical mastitis.

Milk culture. The "gold standard" of a definitive detection of mastitis is based on positive culture of pathogens from aseptically collected milk samples. Although milk culture and bacterial isolation can help to diagnose the pathogens and offers suggestion for treatment, it requires laboratory support, it is time consuming and costly, and thus not practical for an early detection. Another important problem with using milk culture is the occurrence of false negative samples. For these reason is preferable to perform indirect test to assess the IMI status of the gland such as Somatic Cell Count.

Enumeration of somatic cell count

The definitive detection of infected animals relies on positive bacteriological culture from aseptically collected milk samples. Bacteriology has clear limitation related to the need of a laboratory support, the time delays to have a response and the high cost. The enumeration of

somatic cell in dairy sheep is commonly accepted to be an indirect way to determine an infection of the mammary gland (González-Rodríguez et al., 1995; Gonzalo et al., 2002), for this reason a series of test aimed to estimate the SCC are used to detect mastitis. Somatic cell counts (SCC) are accepted as an international standard for measurement of milk quality. The current reference method for enumeration of somatic cells in raw milk is the direct microscopic somatic cell count (DMSCC). However, it needs the training and skill of analysts for the accurate, precise, and reproducible results. Other than that SCC is important as an international standard to measure milk quality and milk hygiene. An accurate estimate of SCC is therefore very important to farmers and to the dairy industry when implementing a mastitis control program and to accomplish requisite for quality milk.

When assessing somatic cell count, in order to have a representative SCC of a full 24 hour periods, samples should be collected at both morning and evening milking times. However, the high hour repeatability and the similarity in SCC level between the two milking time suggest that SCC level could be assessed on the basis of sample collected at only one of the milking (Gonzalo et al., 1994a).

A brief description of the main method used to enumerate somatic cell in sheep milk follows.

Direct microscopy: the direct microscopic somatic cell count (DMSCC) standard method was developed by the National Mastitis Council (1996) and is described in "Standard Methods for the Examination of Dairy Products" (2004). The procedure for performing the DMSCC is as follows: milk (0.01 ml) is spread onto a circular area (1 cm^2) on the surface of a clean glass slide and allowed to dry on a level surface. For counting cells in ewe milk, the dried milk smears are stained for microscopic counting using any one of three stains: Levowitz-Weber, a Canadian modification of the modified Newman-Lampert stain or the pyronin Y- methyl green stain. The

cells are observed on a microscope with an ocular reticle. The reticle has a wide and narrow strip centrally located and perpendicular to each other. The wide strip is used for low cell count milk and the narrow strip for high cell count milk. The number of cells counted in the strips is then multiplied by a conversion factor. The limitations of the method are that is time-consuming and the level of training and experience in reading the slide directly influences final count. Results vary also the basis of the operator fatigue level from prolonged use of the microscope.

The California Mastitis Test (CMT): is a semi-quantitative indirect measure of somatic cells in milk and it can be used as a screening test (Schalm and Noorlander, 1957; Schalm et al., 1971). The CMT test is based on scoring the degree of gel formation of DNA from the somatic cell reacting with the CMT reagent in a paddle. The reagent consist of a detergent (sodium alkyl aryl sulfonate), and a pH indicator (reason for purplish colour). When milk and CMT reagent are mixed in equal amounts, the detergent dissolves the lipids of the cell and nuclear membranes of the somatic cell and release the DNA from the nuclei. The solution of the detergent with the DNA will unite to form a gel. As the number of leucocytes increase, the amount of gel formation will increase. The results are reported as negative if there is no signs of gel formation at all, trace if small amount of gel are noticed tipping the paddle, 1+ if significant amounts of gel are seen, 2+ if the gel clumps in the middle of the paddle when swirled, 3+ if the mixture forms a thick gel. The CMT should be run on foremilk. The lowest leucocytes count is in foremilk, so that if CMT is positive on this fraction, the rest of the milk will be positive. It is not advisable to run the CMT on stripping milk (end of milking), in this fraction the SCC level is higher than foremilk because the leucocytes tend to adhere to fat globules. The CMT score in small ruminants is positively correlated ($r = 0.67$) with SCC and infection status (Ziv et al., 1968; Contreras et al., 1996; González-Rodríguez and Cármenes,

1996; Hueston et al., 1986; Maisi et al., 1987; Fthenakis, 1995; McDougall et al., 2001; Suarez et al., 2002). However, there are contrasting reports on the efficacy of the CMT. Score of CMT ranging from 1+ and 3+ were recommended from different authors to differentiate subclinical mastitis in dairy small ruminants with sensitivity and specificity ranging from 51% and 88%, and from 23% and 98%, respectively (Hueston et al., 1986; Fthenakis, 1995; González-Rodríguez and Cármenes, 1996; McDougall et al., 2001; Suarez et al., 2002; Lafi, 2006). The variation in the cut-off point among studies may be due to methodological differences in the definition of IMI, the calculation of the cut-offs or in the prevalence of infection within the study populations. The CMT was designed as an inexpensive animal-side test, rapid and easy to perform, but rather subjective in its interpretation and despite its fair good sensitivity and specificity it cannot be used for counting the correct SCC, which is the absolute factor for determining the price of milk, because of its low sensitivity and specificity. With all its limitations CMT is useful as a screening test to rapidly and inexpensively identify infected udder halves and hence to select the animals for further culture.

Along with the DMSCC and CMT manual test, automated somatic cell counters have been introduced to electronically enumerate the somatic cells in milk.

Cell Counter: based on electronic particle counting, is a high-speed device for particle size analysis that involves adding a formaldehyde solution to the milk to be examined to fix the somatic cells, and eliminating fat particles by treatment with a lysing solution with an overlapping size range of the cells (Miller et al., 1986; International Dairy Federation 1995a). Particles suspended in a weak electrolyte solution are drawn through a small aperture, separating two electrodes between which an electric current flows. The voltage applied across the aperture creates a "sensing zone". As particles pass through the aperture (or "sensing zone"), they displace their own volume of electrolyte, momentarily increasing the impedance

of the aperture. This change in impedance produces a pulse that is digitally processed in real time. The pulse is directly proportional to the tri-dimensional volume of the particle that produced it. In addition, a metering device is used to draw a known volume of the particle suspension through the aperture; a count of the number of pulses can then yield the concentration of particles in the sample.

Fluoro-opto-electronic counter (Fossomatic): is an electronic method based on the staining or labelling of the cells with a fluorescent dye. The equipment consists of a mixing section and a counting section. In the mixing section the milk sample to test is mixed with a buffer and stained with fluorescent molecules that are absorbed by the nuclear DNA of the somatic cell. In the counting section each stained particle are observed with a fluorescence microscope producing an electrical pulse. These pulses are filtered, amplified and recorded. The intensity of fluorescence emitted from each cell is related to the size of cells. Each fluorescent cell in this volume is counted determining the number of cell/ml. During counting the sample flows (Flow cytometry) through a capillary into the cell where the nuclei are illuminated by the excitation light one by one and their fluorescence is detected. The automation of this process allows to process large numbers of samples per hour. In fluoro-opto-elctronic counting process, somatic cells in sheep milk have a similar appearance to those in cow milk and thus sheep milk may be analyzed under a cow milk calibration (ISO 13366-2:2006, IDF 148-2:2006). When the milk samples to test need to be stored before being processed, the addition of chemical preservative is needed. Chemical preservatives (boric acid, sodium azide, bronopol, potassium dichromate) should be added within 24 hours after sampling. In all cases the milk samples should be kept cool until the addition of the preservative. Many laboratories use the fluoro opto electronic counters, ad it is very important that the method is as much accurate as possible, since milk SCC is used in quality payment schemes. The fluoro opto electronic method

has been standardized for cow milk taking into account the influence of different variation factors such as type of preservative used (Schmidt-Madsen, 1979; Lee et al., 1986; Barcina, et al., 1987; Bertrand, 1996, Ubben et al., 1997), the analytical temperature (Miller et al., 1986), storage condition (Lee et al., 1986; Barkema et al., 1997), or milk age (Kennedy et al., 1982). The influence of these factors in ewe milk has been assessed (Gonzalo et al., 1993, 2003) demonstrating as a whole the efficacy of the fluoro opto electronic method in ewe milk.

DeLaval Cell Counter (DeLaval International AB, Tumba, Sweden): is a portable optical cell counter. The DCC counts somatic cell nuclei stained with the DNA specific fluorescent probe (Propidium Iodide). The milk is collected and the nuclei stained inside a cassette containing small amounts of the fluorescent stain. As little as 60 µl of milk sample is needed for the count. By means of a piston, approx. 1 µl of milk is carried toward a measuring window. The nuclei are then exposed to a LED light source and their fluorescent signals recorded and used to determine the SCC. Once the cassette has been loaded and inserted in the instrument, the counts of somatic cell are shown in the display of the instrument. Advantages of the instrument are that is a battery operated portable device and can be used as an animal-side test, it gives an immediate response (less than one minute). Limitations are its initial high cost, the measuring range (10,000 to 4,000,000 cells/ml) and that is designed for SCC determination in raw bovine milk.

In order to prevent abnormal milk from entering the food chain, biological monitoring of raw milk, which involves analysis of microbial and somatic cells, is essential for milk and dairy quality assurance. Each somatic cell count method has its limitations, DMSCC is time consuming, CMT lacks of specificity (especially for low SCC values), and the FSCC requires expensive equipment. There is the necessity of rapid, economic and accurate methods to assess somatic cell count at farm level.

Selecting Appropriate Testing Methods

The accuracy of a diagnostic test is defined as the ability of a test to distinguish between who has a disease and who does not. Validity has two components: sensitivity and specificity. The *sensitivity* is defined as the ability of the test to identify correctly those who have the disease (true positive). The *specificity* of the test is defined as the ability of the test to identify correctly those who do not have the disease (true negative). When performing a screening test on a population, the positive test includes all those individual who really are infected (true positive) and those who do not have the disease (false positive). When dealing with indirect diagnostic test, such as SCC, where the possible outcome is not binary (i.e. "infected" or "uninfected"), but rather continuous (number of somatic cell per ml), a decision must therefore be made establishing a cut-off level above which the test result is considered as positive and below which a result is considered negative. If the detection of any infection is the aim of the test, then the threshold should be lowered to maximize the sensitivity (SE). On the other hand, the increase in SE, will be associated with a decrease in Specificity (SP) and hence an increase in the number of false positive diagnosis. Increasing the threshold allows to increase the sensitivity (SE) of the test and to correctly identify the true negative, but to miss many of the true infected (low SE). Because of this inverse correlation between SE and SP, the selection of a threshold depends on the reason for the test being performed, whether is used as a screening test or a diagnostic test. On a practical basis what is important is to know what is the probability that the animal is actually infected if the test result is positive. This is the *positive predictive value* (PPV) of the test. The PPV is calculated with the following formula: true positive divided by the number of resulted positive (true positives + false positives). On the other hand, if the test resulted negative, the probability that the animal does not have the

infection is the *negative predictive value* of the test. It is calculated by dividing the number of true negatives by all those who tested negative (true negatives + false negatives).

Positive and negative predictive values are affected by the prevalence of disease in the tested population.

Unfortunately there is no agreement on the SCC threshold to define infected and non infected udder in dairy sheep. The range of SCC proposed in the different studies overlaps. Determination of a threshold is a compromise between sensitivity and specificity (Bergonier et al., 2003). Regardless of what threshold is chosen, there will be some uninfected ewes with SCC above the threshold (false positive results) and some infected ewes with SCC below the threshold (false negative results).

The selection of the threshold depends on the use to which the SCC is being put, and on the cost associated with a wrong decision. If the SCC is used to select ewes for treatment at dry off, the threshold should be lowered to maximize the sensitivity. If SCC are used in culling decision, a higher cut-off should be raised (maximize the specificity) to ensure that only true infected animals are selected. Instead of raising the threshold could be to require 2 or more SCC over the cut-off for a test to be considered “positive”. This is a feasible approach in those regions where monthly test day are performed.

An alternative method would be to use the “likelihood ratios”. Such approach consist to compute the odds of an IMI for a given SCC range. The use of likelihood ratios has two main advantages: a) eliminates the need of a strict cut-off value; b) incorporates information on flock prevalence.

1.4. Dry therapy

Intramammary administration of long-acting antibiotic therapy at dry off (DT) effectiveness has been assessed in dairy cattle, and this practice has been recommended for many years as one of the most effective tools in mastitis control programs in lactating dairy cows (Neave et al., 1966; Natzke, 1981; Berry and Hillerton, 2002). The aim of dry therapy is to cure existing IMI and preventing the onset of new ones at parturition (Postle and Natzke, 1974; Eberhart, 1986). Several studies have assessed efficacy of antibiotic dry treatment (DT) in dairy sheep (De Santis et al., 2001; Chaffer et al., 2003; Gonzalo et al., 2004; Shwimmer et al., 2008; Linage and Gonzalo, 2008). In all these studies the use of DT was associated with reduced prevalence of intramammary infection in the post lambing period. However should be pointed that all the studies were conducted with methodologies not always comparable. Some information is also available on the efficacy of intramammary dry treatment in meat sheep (Hendy et al. 1981; Watson and Buswell, 1984; Ahmad et al., 1992b; McCarthy et al., 1988; Croft et al., 2000). One study performed in North America, evaluated the efficacy of intramammary antibiotic treatment during the dry period of ewes that suckled lambs (Hueston et al., 1989). They reported that untreated ewes were 2.6 times more likely to develop new intramammary infections as compared to ewes that received dry treatment.

Treatment strategies could be based either on a selective dry-off therapy or complete dry-off therapy. In a complete DT strategy (CDT) all glands of animals are treated, whether in the selective dry off therapy (SDT) only infected udders are treated. The glands of the animals requiring antibiotic treatment are selected by a clinical examination or by iSCC (Natzke, 1981; Rindsing et al. 1978). Although CDT and SDT effects on IMI have been well assessed in dairy cows (Eberhart, 1986; Berry & Hillerton, 2002), little information is available on selective intramammary dry treatment in dairy ewes (Gonzalo et al., 1998, 2004). Generalized

intramammary antibiotic therapy should be used in flocks with high prevalence conditions (>50%), whether selective dry-off therapy is preferable when considering some typical conditions of small ruminants (Poutrel et al., 1997). The sheep husbandry system, the larger herd size and the lower income of sheep producer make the necessity of specific strategy for mastitis control. The average treatment cost per animal is higher when compared to the culling value of small ruminants, and when it is combined with the higher number of animal to treat in the complete vs. selective dry off therapy determine further increase in the cost. Another consideration is the high overall self-cure rate that ranges between 35.0 to 67.0% in small ruminants (Watson and Buswell, 1984; Hueston et al., 1989; Paape et al., 2001; Bergonier and Bethelot, 2003). In this scenario SDT approach could be beneficial reducing the cost of antibiotic treatment. Additional advantages of such strategy may be the reduction of discarded milk and of the potential risk of antibiotic residues in milk (Gonzalo et al., 2004). Most of the available treatments are specific for cows. Withdrawal times adopted for cows not necessary valid for ewes. In fact, the patterns of antibiotic excretion are very different between cows and sheep mammary glands (Pengov and Kirbis, 2009), so the withholding time when cow's formulation are used "extralabel" to treat dairy ewes, should be longer than recommended for dairy cows. It is generally accepted that considering the relative long dry off period in dairy ewes the risk of antibiotic residues in the milk could be considered almost null (Bergonier et al., 2003). Lohuis et al. (1995) reported no residues in milk after three days of lambing in ewes treated at dry off with a bovine formulation. A study performed on a group of Sardinian sheep with subclinical mastitis treated with intramammary admistration of at dry off with Cloxacillin showed no risk of residues if the milk withholding period is observed (Marogna et al., 2007). However antibiotic detection methods for sheep need to be standardized (Yamaki et al., 2004; Montero et al., 2005).

Besides general recommendations there are no treatment protocols available for sheep. Few products are registered as officially indicated and authorised for dairy ewes. In the USA there are only 8 drugs approved for intramammary administration (Penicillin, Amplicillin, Ceftiofur, Cephalothin, Novobiocin/Penicillin; Hetacillin, Pen/Streptomycin; Erythromycin & Pirlimycin), but none of these have specific indication for dairy ewes (FDA, 2009). In the European Union (EU) only few intramammary formulation are actually registered for use in dairy ewes. In Italy Cloxacillin have been registered for this aim (De Santis et al., 2001), while in France only one treatment is officially approved by the French Administration for dry ewe intramammary treatment (Longo and Pravieux, 2001). As a general practice, treatment designed for the cows are used to treat mastitis of small ruminants (Shwimmer et al., 2008). Being the majority of treatment formulated for cows, withdrawal times are unknown in sheep, and this pose public health implication related with antibiotic residues in milk. General recommendations available for sheep are intended to ensure strict hygienic conditions during administration: complete milking, scrub teat ends with cotton soaked in alcohol before administration of DT, infusion of a single tube for each half-udder (instead of using the same tube for both halves), partial rather than of complete cannula insertion to avoid teat duct traumatism. After administration of DT, dip teats using a germicidal solution. If these hygienic administrations are not respected there may be the risk of the onset of opportunistic IMI. Mycotic mastitis is usually associated with intramammary infusion of contaminated drugs and infusion syringes (Paine, 1952; Loftsgard and Lindquist, 1960; Mantovani et al., 1970; Farnsworth and Sorensen, 1972; Thompson et al., 1978; Richard et al., 1980; Kirk and Bartlett, 1986; Krukowski et al., 2000). Few cases of mycotic mastitis by *Aspergillus fumigatus* have been reported in small ruminants (Berthelot and Bergonier, 1993; Jensen et al., 1996; Pérez et al., 1998). An association

between mammary aspergillosis and incorrect administration of antibiotic at drying off has been previously observed in dairy ewes (Las Heras et al., 2000).

1.5. Teat sanitation

Teat disinfection with germicidal teat dips is a simple, effective and economical means to reduce bacterial populations on teat skin, and they're commonly used to reduce the rate of new infection among dairy cows (National Mastitis Council, 1996). This practice proved to be effective on reduction of new IMI in dairy cattle (Philpot and Pankey, 1975; Natzke, 1977; Philpot, 1979; Farnsworth, 1980; Pankey et al., 1984; Galton et al. 1986; Rasmussen et al. 1991; Oliver et al. 1993; Nickerson, 2001; Magnusson et al. 2006). A variety of germicides are incorporated into teat dip products such as iodine, chlorhexidine, quaternary ammonium, sodium hypochlorite, dodecyl benzene sulfonic acid, chlorine, nisin, hydrogen peroxide, glycerol monolaurate, and fatty acids. These germicides inactivate bacteria through chemical or biological action (Nickerson, 2001).

Teat disinfection may be conducted immediately after milking and is termed post milking teat disinfection (postdipping), or just before milking and is termed pre milking teat disinfection (predipping).

Postmilking sanitation: the disinfection of teat ends after milking as an aid in mastitis prevention was first considered in 1916 (Moak) and is considered as the single most effective practice for prevention of IMI of lactating dairy cows (Pankey et al., 1984). Postdipping is aimed to control bacterial load on the teat ends immediately after removal of the teatcups to minimize their further spread into the gland. Teat dipping is a preventive measure that reduces the rate of new infections by contagious pathogens (S*taphylococcus aureus* and *Streptococcus agalactiae*) by approximately 50% (Neave et al., 1969; Wesen and Schults, 1970; Schultze and

Smith, 1972; Stewart and Philpot, 1982; Eberhart et al., 1983; Nickerson et al., 1986; Pankey et al., 1984a, Pankey et al., 1984b; Oliver et al., 1989). Postmilking teat sanitation in dairy cattle significantly reduces infections by minor mastitis pathogens such as Coagulase-negative staphylococci (Eberhart et al., 1983; Pankey et al., 1984; Hogan et al., 1987; Pankey and Galton, 1989; Drechsler et al., 1990; Hogan et al., 1990). Not all types of infection are reduced equally by germicidal teat dips, in fact the bactericidal activity of the sanitizer usually last for a short time after dipping (3 or 4 hours), for this reason postdipping sanitation is ineffective on environmental pathogens such as coliform bacteria (*E.coli*) and Streptococci other than *S. agalactiae* (Wesen and Schultz, 1970; Schultze and Smith, 1972; Eberhart and Buckalew, 1972; Natzke, 1977; King, 1981; Eberhart et al., 1983; Smith, 1983; Pankey et al., 1984; Oliver et al., 1991;). The primary sources of environmental pathogens include bedding, manure, soil, and feedstuffs. Therefore, exposure of teats to environmental pathogens continues throughout the milking and intermilking periods. Although most germicidal products kill coliforms and environmental streptococci on the teat skin, exposure to these pathogens occurs primarily between milkings, long after the disinfectants have lost their antibacterial activity (Godinho and Bramley, 1980; Oliver et al., 2001). An additional advantage of postdipping is an improvement of teat skin condition, in fact many sanitizer are formulated with emollient additives (glycerine) which alleviate the irritating effect of germicides (Fox et al., 1991; Fox, 1992). In EU countries Regulation 853/2004 and subsequent amendments stated that teat dips or sprays are used only after authorisation or registration in accordance with the procedures laid down in Directive 98/8/EC concerning the placing of biocidal products on the market. Teat dip falling within the drug definition (if formulated to contain one or more antimicrobial chemicals as active ingredients, and labelling includes claims for controlling mastitis or the spread of mastitis, or labelling includes other claims for controlling microorganisms in or on

the animal) are subject to the registration and drug listing requirements by FDA. Few studies have been carried out on the effectiveness of post milking teat sanitation in dairy ewes, reporting dissimilar results. Teat dipping was very effective in preventing new IMI (Contrearas et al., 2007) while it was ineffective in to restore udder health in sheep with subclinical mastitis (Klinglmair, 2005). The broad application of teat dipping is limited by the perception of dairy farmers that this practice could negatively affect parlor throughput. A possible solution would be represented by the use of teat sanitation just for a limited period, when mastitis outbreak are more likely to happen (i.e. beginning of milking and after weaning) (Bergonier and Berthelot, 2003).

Premilking sanitation. Predipping is practiced before unit attachment and it is intended to combat environmental pathogens upon which post milking disinfection is ineffective. The incidence of IMI is highly correlated to the number of mastitis pathogens on the teat end at milking. Premilking teat sanitation (predipping) was first discussed in 1984 (Bushnell) as a method to reduce microbial population to an acceptable level on teat skin before milking. This would aid in reducing the spread of microorganisms and in minimizing the number of bacteria that can eventually enter the teat canal (Pankey, 1989). The use of premilking teat sanitation has spread with the decrease in mastitis caused by contagious mastitis organisms as a potential method to control environmental pathogens. Premilking teat disinfection with low iodine concentration formulations was effective for the prevention of environmental mastitis (Pankey et al., 1987), especially when in combination with postmilking teat disinfection (Oliver et al., 1993a, 1993b, 1994). Udder infections with Coagulase Negative Staphylococci (CNS) were not controlled by predipping (Pankey et al., 1987; Oliver et al., 1993a; Oliver et al., 1993b; Ruegg and Dohoo, 1997). Predipping with low concentration of iodine proved to reduce

the incidence of IMI by 50 percent or more in cattle (Bushnell, 1984; Galton et al., 1984; Galton et al., 1988; Pankey and Galton, 1989; Blowey and Collis, 1992; Langridge, 1992).

Types of sanitizer. There are many teat sanitizers available but only few have been tested. A teat dip to be considered effective should reduce the bacterial count of 3 or preferably 4 or 5 log (Philpot and Pankey, 1978), while other authors consider a reduction of skin populations by 85% to 90% effective to reduce the incidence of new IMI (Farnsworth, 1980). A good teat dip should meet the following requirement: a) having a broad and rapid bactericidal activity; b) not being irritative to the teat skin; c) do not leave residues in milk; d) being cheap. Iodophors and chlorhexidine-based sanitizer meets most of the requirements.

Iodine compounds: iodine is a broad spectrum germicide and has a rapid effect to kill bacteria and their spores, moulds, yeasts and viruses. Iodine based compounds are referred to as iodophors. The term "iodophor" literally means the iodine carrier. Iodophors are polymeric organic molecules (alcohols, amides, sugars) capable of complexing iodine, resulting in reduced equilibrium concentration of the iodine compared with those of pure aqueous solutions with the same total iodine concentrations (Gottardi, 1991). The effect of iodophors depends on the level of available iodine. The available iodine is present in two forms, the complexed iodine, which is not antimicrobial, and the uncomplexed form (free iodine). The free iodine is the form that provides the antimicrobial activity by oxidizing the microorganisms. The free and the complexed iodine components of the iodophor are in a state of chemical equilibrium. When the free iodine is used up, is immediately replaced from the complexed iodine. Thus, free iodine is always available until the total amount of available iodine in the iodophor is depleted. According to the solvent used to complex the iodine iodophors can be divided into three groups: a) pure aqueous solutions; b) alcoholic solutions; c) iodophoric preparations, which exhibit intrinsic differences in their chemical and microbial properties.

When an iodophor is diluted in water, dispersion of the micelles occurs and most (80-90%) of the iodine becomes slowly liberated as the active form of responsible for its antimicrobial activity. However, aqueous iodine products tend to be toxic or irritative, causing skin sensitization and delay healing of open wounds. Iodophors remain antimicrobially active if the pH does not rise above 4. Commonly iodophors are formulated with phosphoric acid, conferring a pH of about 3. Their potency may be markedly decreased when they are diluted excessively in hard alkaline water. A number of emollients, such as glycerine and lanolin, have been used in teat dip formulations. The addition of emollient (2 to 10%) replaces natural oils lost from the skin and helps to prevent drying, chapping, and irritation. Concentration of emollients of 10 to 15% resulted in a decreased efficiency of iodophor products (Philpot, 1975). Formulation of iodine germicides and the availability of free iodine, have a greater impact than concentration on their effectiveness (Murdough and Pankey, 1993; Foret et al., 2005).

Chlorhexidine: is a halogen, member of the biguanide group of compounds that have a rapid bactericidal activity. Its activity is toward Gram positive and Gram negative bacteria, fungi, but it has no effect on viruses or spores. To be effective, chlorine-based teat dips must be used within several hours of preparation because of short shelf life. Unlike iodophors, chlorhexidine is more active at alkaline than at acidic pH and its efficacy is greatly reduced in the presence of organic materials. This disinfectant is used where there is concern over the iodine residues in milk. Teat sanitizers using this germicide contain between 0.35 to 0.55% chlorhexidine gluconate or acetate as well as humectants and emollients to minimize irritation.

There are several arguments regarding the use of teat sanitation as a milking routine. The dipping with iodine products increases the risk of iodine residues in milk (Galton et al., 1986b). Contamination may be by absorption through the teat skin or aspiration of residual iodine left

on the teat surface by the preparation process (Conrad and Hemken, 1978). When the same predipping solution is used frequently, it would become contaminated by organic matter and may contain resistant mastitis pathogens. Such contamination might have the risk of transfer of pathogens among animals. It is therefore recommended to use fresh solution at each milking. Galton et al., 1986, showed that, provided the teat are adequately wiped afterward, premilking dipping with a 0.1% iodophor dip had no significant effect on milk iodine levels, but that 0.5% iodophor preparation led to increases in milk iodine. Other study (Aumont, 1987) concluded that post milking teat disinfection with 0.5% iodophor produced only a small increase in milk iodine. Accurate drying of teats with paper towels after predipping is needed to reduce iodine residue in milk (Galton et al., 1984; Galton et al. 1986; Rasmussen et al., 1991; Ruegg, 2004), and is a recommended practice (National Mastitis Council, 2004).

The conventional method for applying teat dips is to immerse teats using some type of cup that contains the teat dip. If this type of applicator is not kept clean and becomes heavily contaminated with organic material, spread of mastitis causing organisms from animal to animal is possible. However, contamination of teat dips with mastitis pathogens during the course of a milking is highly unlikely if the product is an effective germicide and the dip cup is not grossly contaminated.

1.6. Thesis project

Recent food safety incidents have heightened consumers awareness on risk related to foodstuff of animal origin and, as a consequence, the demand of assurance of a high level of protection of human health. The consumption of fluid milk and milk products, especially from raw milk, has been reported to be associated with public health problems/ foodborne disease (Headrick et al., 1998). Considering than more than 90% of all reported cases of milkborne

disease is of bacterial origin (Bean et al., 1996), the evaluation of the microbiological quality of milk is of central importance in order to achieve a reduction of the risk of foodborne disease.

Sheep's milk is mainly used for cheese making and yogurt production. This type of production has a long tradition in many countries in Europe, especially around the Mediterranean basin, Middle East and North Africa (Berger et al., 2004; Pirisi et al. 2007), and is becoming very popular in the last few years in the USA (Berger et al., 2004; Thomas and Haenlein, 2004). Since most sheep's milk products are made from raw milk animal and udder health is the most important prerequisite to produce hygienic milk, it is clear how the production of quality milk depends on a great amount the presence of udder infections. Mastitis, particularly in its subclinical form, has a great impact on milk hygiene. The presence of microorganism in milk and milk products has important implications on food safety, quality, regulations and public health. Mastitis is considered to be the disease that has the greatest financial impact on the dairy industry. The potential economic losses of mastitis in dairy ewes include treatment costs, premature culling (Watson and Buswell, 1984; Saratsis et al., 1998; Bergonier and Berthelot, 2003), reduced milk yield, changes in milk composition (Schalm et al., 1971; Torres-Hernandez and Hohenboken, 1979; McCarthy et al., 1988; Burriel, 1997; Leitner et al., 2004) and reduced lamb performance (Fthenakis and Jones, 1990; Keisler et al., 1992; Moroni et al., 2007). For regions with quality payment systems, reduced milk premiums and reduction in animal welfare can be additional consequences (Pirisi et. al, 2007; Barilett et al., 2001). A recent study found that sheep milk with a SCC>1,000,000 decreased the cheese yield and increased the development of rancid flavours in the cheese (Jaeggi, 2003).

Mastitis has also implication on the quality of milk. Milk quality is a general term which includes a two-folded aspect: composition quality (physical and chemical) and hygienic quality. Some of the criteria for evaluate hygienic quality of milk are bacterial count and somatic cell

count and antimicrobial drug residues. The bacterial count refers to the number of aerobic microorganisms that growth at 30°C. There are different limits established in different countries for sheep raw milk. In Europe, the Regulation EC n. 853/2004, on the hygiene of food of animal origin, covers legal requirements to be respected for raw milk. EU limits set the bacterial count limit of no more than 1,500,000 cfu/ml. Although the EU defines really specific parameters for SCC in cow's milk (no more than 400,000 cells/ml), there is no legal limit for SCC in small ruminants. In other countries, such as USA, the U.S. Grade "A" Pasteurized Milk Ordinance (PMO) requires sheep milk at the farm to have the same limits established for cow milk, bacterial count of not more than 100,000/ml of milk and somatic cell count of not more than 750,000/ml of milk. The hygienic implications of mastitis are related to the risk of infection or intoxication by pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., etc.

Mastitis, particularly in their subclinical form, serve as reservoir of pathogens that can be shed into the milk and constitute a potential risk for human health. The SCC is a useful predictor of IMI in dairy ewes (González-Rodríguez et al., 1995; Barillet et al., 2001; Gonzalo et al., 2002) and while SCC is not a public health concern itself, bulk tank milk SCC (BTSCC) constitute an indicator of the general state of udder health in a dairy sheep flock and can be used as an indication of hygienic milk and to improve safety of dairy products. It has also economical implications, where systems of quality payment are implemented, SCC is one of the parameter considered for applying bonus or penalty. Mastitis control and, as a consequence, the production of quality milk is the goal of sheep-breeding organization and dairy farmers. The control of SCC with the application of appropriate programs has been largely studied in dairy cows. In dairy small ruminants only general recommendations rather than exhaustive protocols are available, and in most of the cases extrapolated from researches conducted for

dairy cattle. The quality of raw milk is a concern of dairy farmers, processor, and consumers and the adoption of a mastitis control program is essential in order to avoid the introduction pathogenic agents into the milk prevent the risk of foodborne disease in the dairy food chain. A complete control program should include indication for: a) correct diagnosis of mastitis; b) treatment; c) prevention.

The diagnosis of mastitis could be conducted by a clinical exam, bacteriological culture or the enumeration of somatic cells. The somatic cells are normally present in the milk, and their count (SCC) is an indicator of udder health and is often used as a predictor of intramammary infection (González-Rodríguez et al., 1995). The efficacy of automated somatic cell counter such as Fossomatic (FSCC) has been proven on ewe milk when compared with the direct microscopic (DMSCC) reference method (Gonzalo et al., 2003). At farm level the use of a portable device such as the DeLaval cell counter (DCC; DeLaval International AB, Tumba, Sweden), would be a useful tool for mastitis control strategies (Gonzalo et al., 2006). In **Chapter 2** is described a study aimed to compare the correlation between DCC and FSCC methods on ovine milk.

Treatment of mastitis with administration of intramammary long-acting antibiotic therapy at dry off (DT) is one of the most effective tools for mastitis control in lactating dairy cows. The effectiveness of DT has been assessed in dairy cows (Natzke, 1981) and several studies have assessed efficacy of antibiotic dry treatment (DT) in dairy sheep (De Santis et al., 2001; Chaffer et al., 2003; Gonzalo et al., 2004). In all these studies the use of DT was associated with reduced prevalence of intramammary infection in the post lambing period. In **Chapter 3** are presented the result of study 2 whose objective was to determine the effect of intramammary antibiotic dry treatment given to milking ewes on production, prevalence of intramammary infection and somatic cell count in the subsequent lactation.

Prevention of mastitis. Teat disinfection is a practice meant to decrease the risk of intramammary infection by reducing bacteria population on teat skin (Bramley et al., 1996). When disinfection is applied before the attachment of teatcups (pre-dipping) it is intended to reduce the infection due to environmental pathogens (Weihuan and Pyörälä, 1995). Although in dairy cows pre-dipping proved to be effective in reducing bacterial load, on teats skin before milking and in preventing environmental mastitis (Galton et al. 1988; Oliver et al., 1993; Skrzypek et al., 2004), no information is available for dairy ewes. The **Chapter 4** is a study aimed to assess the impact of premilking teat sanitation on somatic cell count and milk production in dairy ewes. In **Chapter 5** are presented the results of the combined effect of dry treatment and teat sanitation on SCC and milk production in dairy ewes.

Study site

All the study were carried out at the University of Wisconsin, Madison dairy sheep research facility, located at Spooner in northwest Wisconsin (45°49' N and 91°52' W), which is the only University sheep research station in North America. The facility is operative since 1996. Seasonal milking is the system adopted with lambing concentrated over a few weeks and the majority of ewes milking at more or less the same time. The milking is performed in a double twelve indexing stanchion parlor with high-line pipeline and six milking units. The throughput is 150 ewes milked per hour. The indexing stanchions are equipped with a feed hopper, lock-in head gate, and a rollback system. The pit is centrally located and approximately 80 cm deep. The parlor is equipped with Alfa-Laval Agri milking machine. An electronic pulsator control panel allows to change the pulsation rate of 60, 90, 120, or 180 per minute and a ratio of 1:1 or 2:1. During the period of this study, the milking vacuum was 38 Kpa, the pulsation rate was 180/minute and the pulsation ratio was set at 50 milk to 50 rest. The ewes enter the parlor twelve a time and when each ewe takes its place, the stanchion is rolled back. The six milking

units are attached on alternate ewes on one side. Once the milk is completed the unit are manually removed and attached to the next ewes. When the 12 ewes of one side are milked, the milking unit are swung to the other side of the pit, and the ewes are released. Neither udder washing nor teat disinfection is performed before unit attachment. After milking teat are immersed in an iodine germicide solution. Individual milk production is recorded monthly using the DHIA Waikato milk meter jar. Regular bulk tank samples are sent to a certified laboratory for checks on bacteria, somatic cell count, drug residue, and sediment.

During the time study, the day one weaning system was adopted, where lambs are removed from their dams within 24 hours after birth and raised on artificial milk replacers or with part of the milk collected from the ewes. The ewes are machine-milked twice daily for the entire lactation. Lambing occurred in mid January and ewes are milked until late fall (September-October). Sheep are fed alfalfa hay silage (November to May), or graze pastures (mixture of Orchard grass and Kura clover) (May to October). In 2007 the flock consisted of 331 milking ewes (245 multiparous ewes and 86 primiparous ewe lamb) with breed varieties including East Friesian, Lacaune, East Friesian-Lacaune crossbreeds and crossbreds with meat ewes. Ewes ranged in age from 18 months to 8 years of age (1st to 7th parity). The milking period was 166 and 209 days for first lactation and mature ewes, respectively, and the average milk production was 1.34 kg per ewe per day for first lactation ewes/ewe lambs and 1.68 kg per ewe per day for multiparous ewes.

Chapter 2
Performance of the direct cell counter on ovine milk samples

2.1. Introduction

Somatic cell are used as an indication of udder health (González-Rodríguez et al., 1995; Barillet et al., 2001; Gonzalo et al., 2002) and its measure is becoming one of the main parameter to determine milk quality and the price of raw milk within the dairy industry (Pirisi et al., 2007). Individual SCC (iSCC) is a useful predictor of infected gland, though there is no accepted threshold that can permit to differentiate between "healthy" and "infected" udders (Green, 1984; Mackie and Rodgers, 1986; Maisi et al., 1987; Fthenakis et al., 1991; González-Rodríguez et al., 1995; Mavrogenis et al., 1995; Fthenakis, 1996; Pengov, 2001; McDougall et al., 2001; Berthelot et al., 2006). The bulk tank SCC (BTSCC) allows to predict the prevalence of mastitis at flock level (Lagriffoul et al., 1999). A strong relationship (R^2 = 0.79) has been demonstrated between BTSCC and the estimated prevalence of infected animal in a flock. Berthelot et al. (2006) estimated a 2.5% increase in prevalence of infected ewes with an increase of 100,000 cells/ml in the bulk tank. The reference method for the enumeration of somatic cells is the microscopic method, recommended by the ISO 13366-1/IDF 148-1 (2008). The principle of the test is to spread a smear of the milk to test on a slide, stain the cell with a dyeing solution and then to count the stained cells using a microscope. The number of cell counted is multiplied by a conversion factor. The limits of the direct microscopy are that is time consuming and requires trained staff. Other method to enumerate somatic cells should be assessed using the direct microscopy as a "gold standard". Local or national dairy laboratories process large numbers of milk samples a time, so automated and reliable system are required to enumerate somatic cells. The use of automated fluoro-opto-electronic somatic cell counter such as the Fossomatic™, has been well standardized for bovine and ewe milk and it compares favourably with the reference method (Heald et al., 1977; Schmidt-Madsen, 1979; Miller et al., 1986;

Barcina, et al., 1987; Bertrand, 1996; Barkema et al., 1997; Gonzalo et al., 2003, 2004). The Fossomatic™ is an electronic method based on the staining or labelling of the cells with a fluorescent dye. The equipment consists of a mixing section and a counting section. In the mixing section the milk sample to test is mixed with a buffer and stained with fluorescent molecules that are absorbed by the nuclear DNA of the somatic cell. In the counting section each stained particle are observed with a fluorescence microscope producing an electrical pulse. These pulses are filtered, amplified and recorded. The intensity of fluorescence emitted from each cell is related to the size of cells. Each fluorescent cell in this volume is counted determining the number of cell/ml. During counting the sample is forced to flow (Flow cytometry) through a capillary into the cell where the nuclei are illuminated by the excitation light one by one and their fluorescence is detected. The automation of this process allows to process large numbers of samples per hour. In the fluoro-opto-elctronic counting process, somatic cells in sheep milk have a similar appearance to those in cow milk and thus sheep milk may be analysed under a cow milk calibration (ISO 13366-2:2006, IDF 148-2:2006). The performance of Fossomatic method has been evaluated in sheep milk by some authors (Gonzalo et al., 1993), and its optimal analytical conditions (type of preservation, analytical temperature, and milk age) have been defined for refrigerated and stored at ambient temperature milk (Gonzalo et al., 2003). The Fossomatic™ FC requires cumbersome and expensive equipment and should be calibrated regularly using standard solutions that have been confirmed by DMSCC for quality control. Both methods (direct microscopy and Fossomatic™) need a laboratory support, and though they're reliable and give an accurate enumeration of the somatic cell, they cannot be used at farm level. The implementation of somatic cell control strategies is limited at farm level by three main order of factor: the cost, the time and the accuracy of the test. The California Mastitis Test (CMT) is a rapid, inexpensive

animal-side test (Schalm and Noorlander, 1957; Schalm et al., 1971). The CMT operates by disrupting the cell membrane of somatic cells present in the milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel. The thicker is the gel the higher is the DNA (and thus cells) contents. Due to its low specificity and sensitivity CMT is suitable only as a screening test (Hueston et al., 1986; Fthenakis, 1995; González-Rodríguez and Cármenes, 1996; McDougall et al., 2001; Suarez et al., 2002; Lafi, 2006). The DeLaval Somatic Cell Counter-DCC (DeLaval International AB, Tumba, Sweden) is a portable optical cell counter. The DCC counts somatic cell nuclei stained with the DNA specific fluorescent probe (Propidium Iodide). The milk is collected and the nuclei stained inside a cassette containing small amounts of the fluorescent stain. As little as 60 µl of milk sample is needed for the count. By means of a piston, approx. 1 µl of milk is carried toward a measuring window. The nuclei are then exposed to a LED light source and their fluorescent signals recorded and used to determine the SCC. Once the cassette has been loaded and inserted in the instrument, the counts of somatic cell are shown in the display of the instrument. Advantages of the instrument are that is a battery operated portable device and can be used as an animal-side test, it gives an immediate response (less than one minute). The DCC provides farmers with real time information on udder health and milk quality of their flock. Limitations are its initial high cost, the measuring range (10,000 to 4,000,000 cells/ml) and that is designed for SCC determination in raw bovine milk. Some differences exist between cow and sheep milk. Ewe milk has higher total solids content (butterfat and protein) and that might interfere with cell stimulation and cell pulse emission and produce smaller SCC values (Gonzalo et al., 2006). This differences in sheep milk, calls for specifics operative condition in the use of automated cell counter. Milk dilution with PBS containing fluorescent stains (ethidium bromide or propidium iodide) proved to optimize the accuracy of the DCC (Gonzalo et al., 2006). Allowing a “soaking time” (staying of the milk

inside the cassette before the reading) of 1 or 2 minutes is another operative condition that improves the accuracy of the method (Gonzalo et al., 2008). This additional time could be needed to complete the cell nuclei staining allowing the use of electronic particle counter for ewe milk. This study was conducted in Spain on 2 local dairy breeds (Churra and Assaf breeds) from composite milk samples.

The objective of this study was to evaluate SCC values determined using the DCC for ovine milk obtained from dairy sheep in the U.S.

2.2. Materials and methods

2.2.1. Flock

The study was carried out at the University of Wisconsin, Madison dairy sheep research facility, located at Spooner in northwest Wisconsin (45°49' N and 91°52' W), which is the only University sheep research station in North America. The flock consists of 331 milking ewes (245 multiparous ewes and 86 primiparous ewe lamb) with breed varieties including East Friesian, Lacaune, East Friesian-Lacaune crossbreeds and crossbreds with meat ewes. Lambing occurs in mid January and ewes are milked until late fall (September-October). Sheep are fed alfalfa hay silage (November to May), or graze pastures (mixture of Orchard grass and Kura clover) (May to October). Milking is performed in 24 stall parlor with a high pipe line. During the period of this study, the milking vacuum was 38 Kpa, the pulsation rate was 180/minute and the pulsation ratio was set at 50 milk to 50 rest. During the 2007 lactation period average milk production was 1.34 kg per ewe per day for first lactation ewes/ewe lambs and 1.68 kg per ewe per day for multiparous ewes. The milking period was 166 and 209 days for first

lactation and mature ewes, respectively. Ewes ranged in age from 18 months to 8 years of age (1st to 7th parity).

2.2.2. Samples collection

Milk samples were collected by study personnel during a single farm visit. Half udder milk samples (n = 100) were collected from 50 dairy ewes. A convenience sample with haphazard (pseudorandom selection) was used. After the removal of 3 strips of foremilk, the teats were wiped with cotton balls soaked in 70% alcohol, and 25 ml of milk were manually expressed from each udder into separate collecting tube. Each tube was labelled including date, sheep ID and half udder sampled. Data on milk production were recorded. After collection, samples were divided into two aliquots. One aliquot was processed immediately using the DCC. The DCC cassettes were filled according to manufacturer instructions and allowed to soak for two minutes before insertion of the cassettes into the meter for counting (Gonzalo et al., 2008). The second aliquot of milk was preserved using bronopol and refrigerated until submitted to the local DHIA laboratory where analysis for SCC was performed using a Fossomatic.

2.2.3. Analysis

In order to account for the measuring range of the DCC (10,000 to 4,000,000 cells/ml), FSCC counts outside of this range were truncated at those values. A preliminary analysis was conducted on the raw SCC to check for normality. As expected the distribution was not normal and then, in order to normalize the distributions, SCC data were transformed using Log10 before analysis (Figure 1). To test the relationship between logDCC and LogFSCC a correlation analysis was performed (CORR procedure; SAS vers. 9.1). A mixed model (MIXED procedure; SAS vers. 9.1) was carried out to adjust for the effect of parity and milk yield.

The model used was:

$Ypts = \mu + M + Y + P + e$

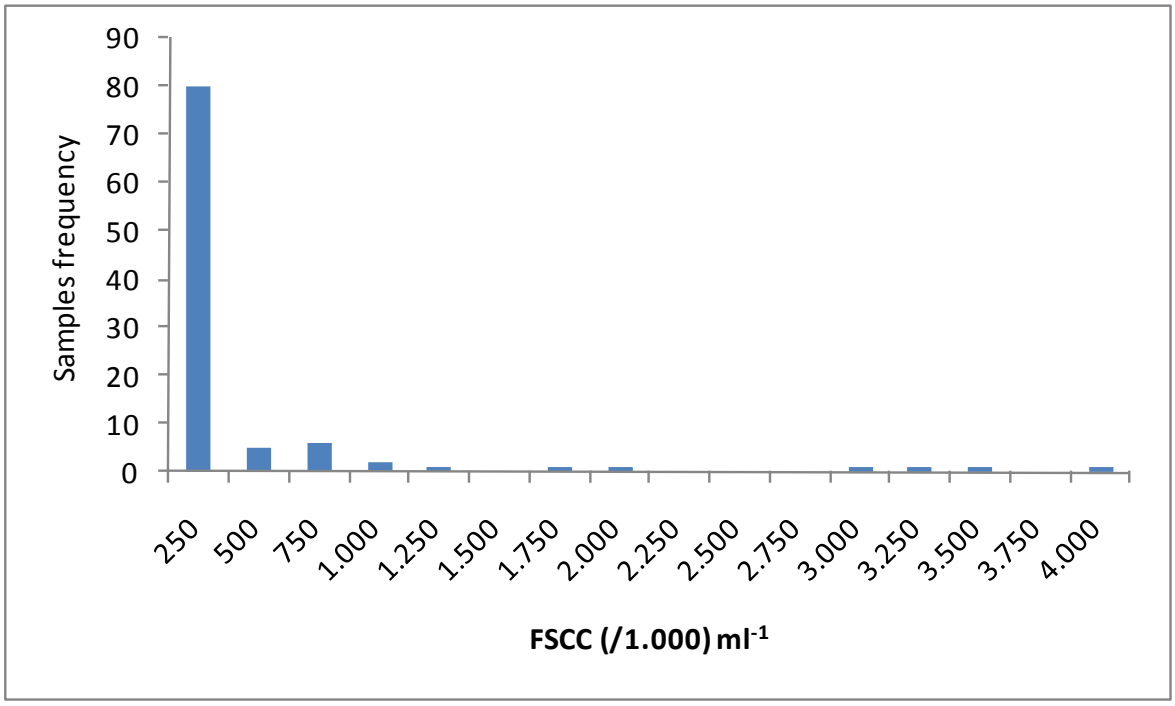
Where (Y) was the response variable $\log_{10}$ SCC , μ is the overall mean, M was the fixed effect of the method (DCC and FSCC), Y was the effect of milk yield, P was the fixed effect of parity, *e* was the error.

2.3. Results

The geometric mean SCC was 144,056 cells/ml and 89,7501cells/ml for the DCC and FSCC, respectively. The mean $\log_{10}$DCC was 5.1, and the mean $\log_{10}$FSCC was 4.9. The coefficient of correlation (r) between FSCC and DCC was 0.94, and the coefficient of determination (R^2) was 0.88 (Figure 2). The ewes enrolled in the study were 1^{st} to 3^{rd} parity and the mean milk production was 0.68 kg. Parity and milk production had no significant effect on the LogDCC (*P* = 0.90 and *P* = 0.50, respectively).

Figure 1a. Distribution of Fossomatic SCC. A) geometric mean; B) log10 transformed data.

A)



B)

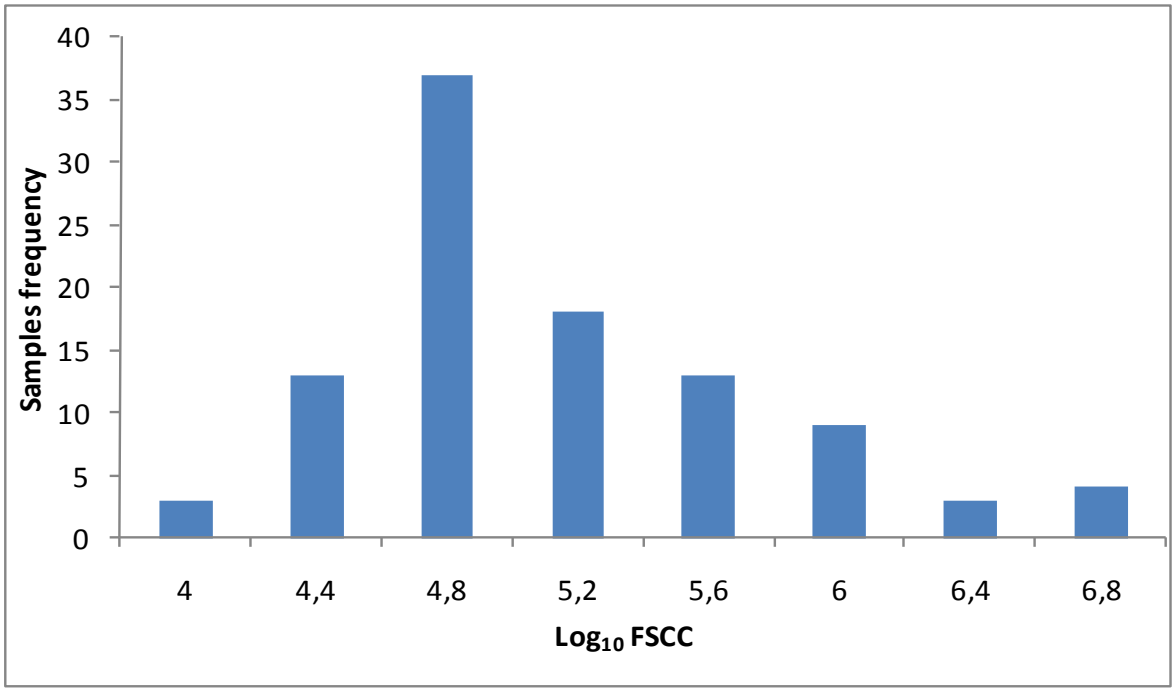
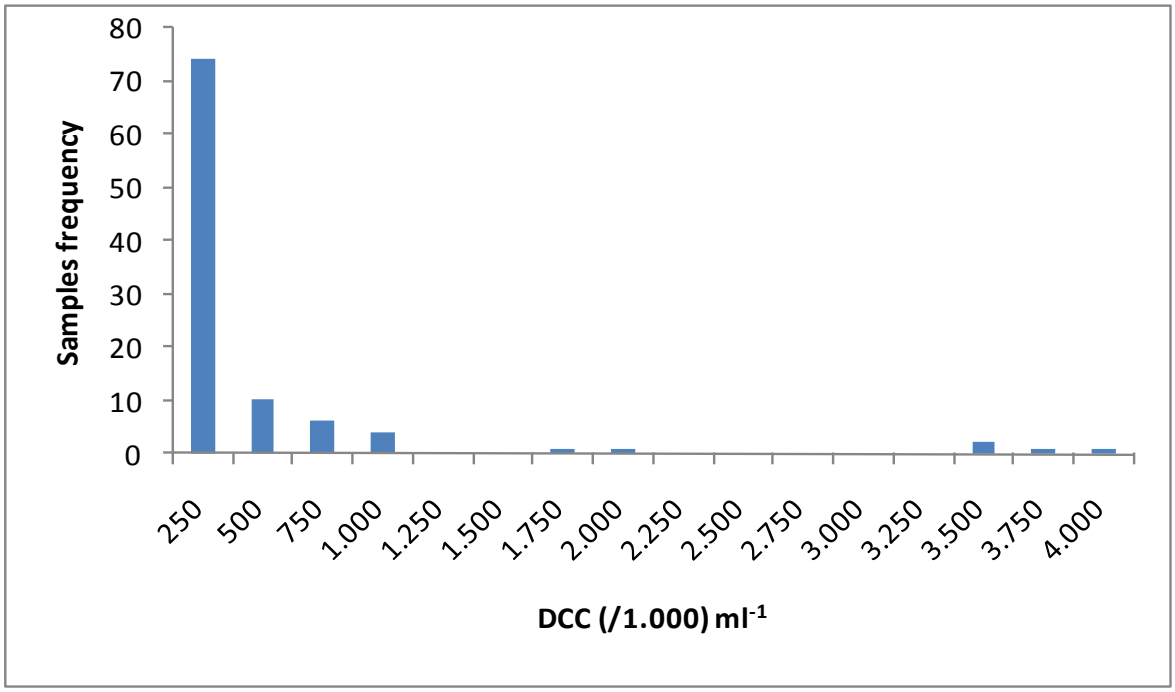


Figure 1b. Distribution of DeLaval SCC. A) geometric mean; B) log10 transformed data.

A)



B)

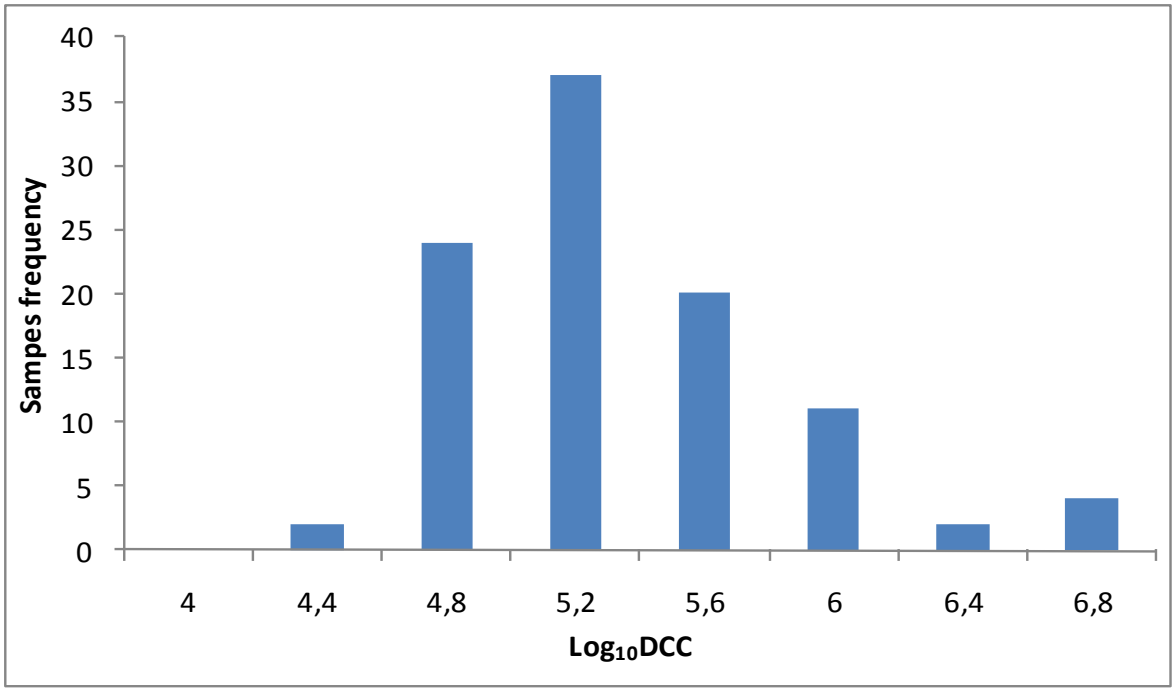
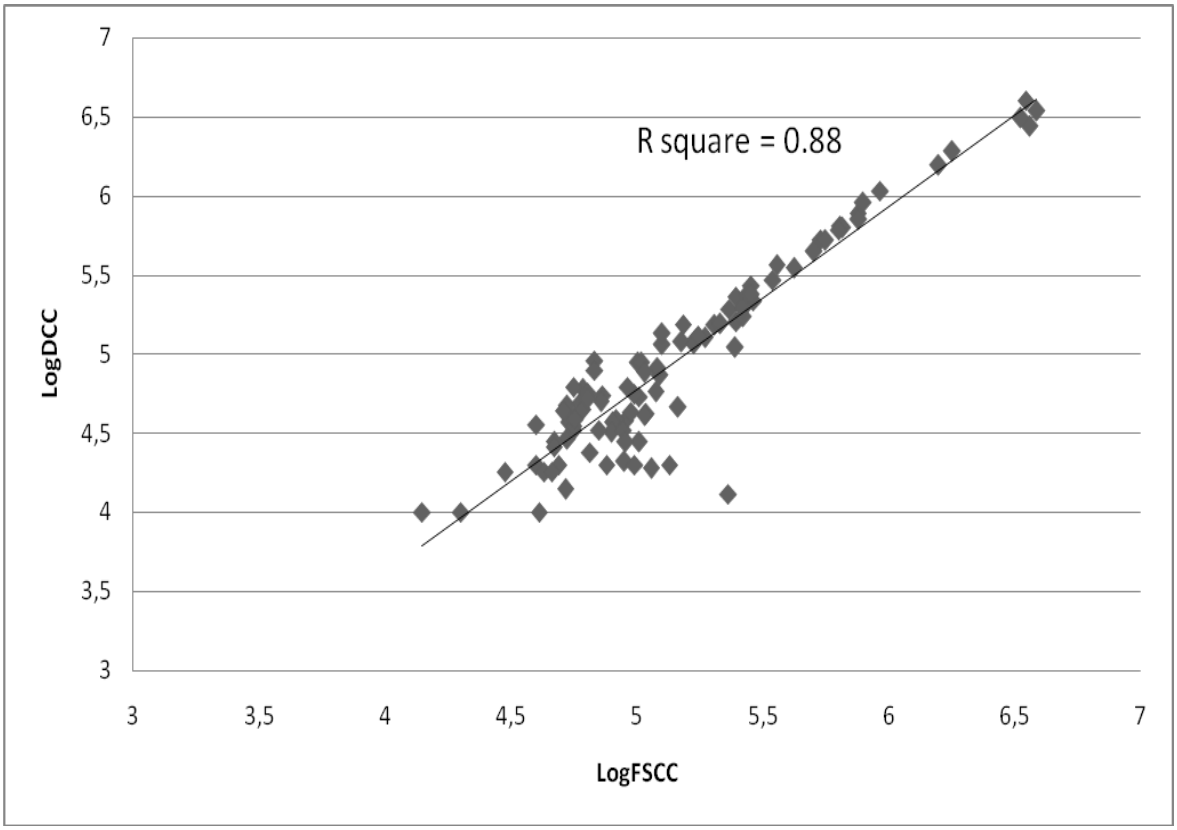


Figure 2. Correlation between LogDCC and LogFSCC.



2.4. Discussion

Milk quality, with respect of hygiene, is an important aspect in dairy sheep farming. Where quality payment systems are applied, SCC is one of the parameters considered when determining premiums or penalties on milk price (Pirisi et al., 2007). As a consequence, the somatic cell count has a great impact on the economy of the farmer and of the dairy industry. Monitoring BTSCC and iSCC, is a fundamental practice in a mastitis control program. Among the methods commonly used to assess SCC are: the direct microscopy (DMSCC), the Fossomatic (FSCC) and the California Mastitis Test (CMT). The DMSCC is the reference method, but has the limitation

that is time-consuming and needs trained and experienced operator. The FSCC is an automated system that allows to process many samples at time, but requires a laboratory support. In countries were the dairy sheep industry is well developed, milk recording on SCC are available on a monthly basis. The reports supplied aid the farmers in monitoring and controlling milk quality in their flock. However, in the period of time between two consecutive test cases of subclinical mastitis might go undetected. A practical, economic and cheap method is needed at farmer level to be an effective tool to control milk quality. The CMT fulfil these requirement, but lacks in objectivity, and though is a useful system in mastitis control, it cannot be used for counting the correct SCC. The DCC has been proposed as an on farm instrument designed for dairy cows. It is a reliable and fast method to enumerate somatic cells. Previous studies conducted in Spain on Churra and Assaf ewes (Gonzalo, et al., 2006, 2008) demonstrated an acceptable overall accuracy, under particular operative conditions, for sheep's milk. Our results are in agreement and demonstrate that the DCC can be an efficient and accurate method for on farm enumeration of somatic cells also in ovine milk of Lacune and Eastern Friesian breeds reared in the USA. The measuring range of the DCC (10,000 to 4,000,000 cells/m) is narrower than other methods, however, considering that most decision are based on SCC values within this range, the use of a portable SCC such as the DeLaval Cell counter, is potentially beneficial for mastitis control programs. A limitation of the method could be the initial high cost of the device and the running cost of the cassettes. The use of the DCC is suggested in all the situation where a prompt response is needed and the time to send the milk sample to a laboratory would make the test results not useful to decide what action should be taken. In conclusion, the DCC is a reliable and rapid method but its cost benefit evaluation is a farmer responsibility.

Chapter 3
Effect of dry treatment on mastitis in dairy ewes

3.1. Introduction

Mastitis is defined as inflammation of the mammary gland (Schalm et al., 1971) and is considered to be the disease that has the greatest financial impact on the dairy industry. Mastitis can cause visual changes in the milk or udder (clinical mastitis), or be diagnosed by increased numbers of somatic cells or by the presence of pathogens in the milk (subclinical mastitis). The potential economic losses of mastitis in dairy ewes include treatment costs, premature culling (Watson and Buswell, 1984; Saratsis et al., 1998; Bergonier and Berthelot, 2003), reduced milk yield, changes in milk composition (Schalm et al., 1971; Torres-Hernandez and Hohenboken, 1979; McCarthy et al., 1988; Burriel, 1997; Leitner et al., 2004) and reduced lamb performance (Fthenakis and Jones, 1990; Keisler et al., 1992; Moroni et al., 2007). For regions with quality payment systems, reduced milk premiums and reduction in animal welfare can be additional consequences (Pirisi et. al, 2007; Barilett et al., 2001).

Somatic cell count (SCC) is an indicator of udder health and it is an indirect method used to detect subclinical mastitis in dairy cows and sheep. Somatic cell counts are considered to be effective for diagnosing intramammary infections in dairy sheep (Gonzalo et al., 1994; Gonzáles-Rodríguez et al., 1995; Pengov, 2001).

Mastitis of small ruminants used for dairy purposes is usually subclinical and is most commonly of bacterial origin. In dairy ewes, subclinical infection can cause severe damage to udder tissues (Burriel, 1997), and may result in significant losses of milk yield and changes in milk composition (Gonzalo et al., 2002; Leitner et al., 2004). In dairy sheep, most cases of mastitis are attributed to staphylococci (Bergonier et al., 2003). Coagulase Negative Staphylococci (CNS) have been frequently reported to be the most commonly isolated pathogens recovered from cases of

subclinical mastitis of dairy ewes (Fthenakis, 1994; Burriel, 1997; Lafi et al., 1998; Ariznabarreta et al., 2002; Gonzalo et al., 2002; Hariharan et al., 2004). Subclinical infection caused by CNS and other mammary pathogens have been associated with increased SCC (Pengov, 2001; Ariznabarreta et al., 2002).

Intramammary administration of long-acting antibiotic therapy at dry off (DT) is one of the most effective tools for mastitis control in lactating dairy cows. The effectiveness of DT has been assessed in dairy cows (Natzke, 1981) and several studies have assessed efficacy of antibiotic dry treatment (DT) in dairy sheep (De Santis et al., 2001; Chaffer et al., 2003; Gonzalo et al., 2004). In all these studies the use of DT was associated with reduced prevalence of intramammary infection in the post lambing period.

One study performed in North America, evaluated the efficacy of intramammary antibiotic treatment during the dry period in meat sheep (Hueston et al., 1989). They reported that untreated ewes were 2.6 times more likely to develop new intramammary infections as compared to ewes that received dry treatment.

There are many factors to consider when evaluating comprehensive use of DT. The first consideration is on the expected bacteriological cure rate. Literature reports cure rates ranging from 50% to 96% (Hueston et al., 1989; Ahmad et al., 1992a; De Santis et al., 2001; Chaffer et al., 2003). Should be pointed that the assessment of “cure” varies between experiments. Differences in cure rate may depend, apart from experimental designs, by the microorganism target of the antibiotic therapy. Prevention of new IMI is difficult to estimate since few data are available on the incidence of subclinical mastitis in dairy ewes. The self-cure should be taken into account, it is

generally considered to be ranging between 35.0 and 67.0% in small ruminants (Watson and Buswell, 1984; Hueston et al., 1989; Paape et al., 2001; Bergonier and Bethelot, 2003). The cost benefit ratio includes a careful evaluation of the risk related to treatment such as the risk of iatrogenic contamination and the risk of antibiotic residues in the milk. Antibiotic treatments require veterinary supervision in order to ensure hygiene during administration. Cases of mycotic mastitis have been reported as consequence of incorrect antibiotic administration (Las Heras et al., 2000). The risk of antibiotic residues in milk is considered almost null by some authors (Lohuis et al., 1995; Bergonier et al., 2003), provided that the withholding time is respected. An accurate evaluation of the cost and benefit of comprehensive use of DT should be based considering all these factors. It is usually recommended to perform a complete dry off therapy in flocks with a high prevalence of IMI (≥50%). The objective of this study was to determine the effect of intramammary antibiotic dry treatment given to milking ewes on prevalence of intramammary infection and somatic cell count in the subsequent lactation.

Some authors (Gonzalo et al., 2004) proposed a selective dry off treatment instead a complete dry therapy. In a selective dry therapy strategy only the infected udders are treated. However in the Spanish study the diagnosis of infection was bacteriological. As the authors pointed out, on a practical basis, and indirect method of detection, such as SCC, is needed for an effective implementation of selective therapies, provided that its sensitivity and specificity are improved. In countries where the dairy sector is well developed, farmers are supplied by improvement agency or private laboratories with monthly report on bulk tank and individual animal somatic cell count. Monthly test day SCC is an important tool that could be used to select the animals that need to be

treated. The aim of this study was to evaluate the efficacy of an intramammary dry off treatment based on the test day SCC as a decision criterion.

3.2. Materials and methods

The study was carried out at the University of Wisconsin-Madison dairy sheep research facility. The flock consists of 331 milking ewes (245 multiparous ewes and 86 primiparous ewes). Lambing begins in mid January and ewes are milked until late fall (September-October). The mean lactation length was 166 and 209 days for primiparous and multiparous ewes, respectively. Ewes ranged in age from 18 months to 8 years of age (1^{st} to 7^{th} parity).

3.2.1. Allocation to groups & administration of treatments

At the end of the lactation season, eligible milking ewes (n = 245) were blocked on the basis of the final test SCC day, and randomly allocated to two treatment groups. The SCC blocks were ewes with the last monthly SCC greater than 400,000/ml (High SCC) or ewes with last monthly SCC less than 400,000/ml (Low SCC). During the final milking session, ewes were randomly assigned within SCC block to receive either an intramammary infusion of 300 mg cephapirin benzathine (DT) or no dry treatment (NT). Teat ends were scrubbed with cotton soaked in 70% isopropyl alcohol, and an entire intramammary tube was administered in each half udder. After administration of DT, teats were dipped using a germicidal teat dip (1% iodine solution). The effectiveness of the allocation process and the retention in the study was evaluated using chi square analysis (PROC FREQ vers. 9.1) and t-test (PROC TTEST; SAS vers. 9.1).

3.2.2. Sampling & Data Collection

The dry period varied from 100-150 days, and lambing occurred in late January through March 2008. After birth, lambs were allowed to suckle for two days, then removed and raised on milk replacer. The ewes were milked twice daily. After lambs were removed and before the first milking parlour milking, trained farm personnel collected a single half udder milk sample from each ewe. The samples were frozen and shipped each week to the UW Milk Quality Laboratory for bacteriological examination. After 14-21 days post lambing, study personnel visited the farm to collect duplicate half udder milk samples (follow-up sample). All milk samples were collected according to NMC procedures (NMC, 1999). One sample was used for bacteriology and the other one to assess the half-udder SCC using a portable somatic cell counter (DCC; DeLaval International AB, Tumba, Sweden).

3.2.3. Bacteriology

Microbiological procedures were conducted according to NMC guidelines (NMC, 1999). Calibrated, 100 µl sterile disposable plastic loops were used to inoculate blood and MacConkey agar plates (Difco Laboratory, Detroit, MI). Inoculated plates were incubated aerobically at 37°C and examined after 24 and 48 h. Samples were also screened for *Mycoplasma* species using comingled milk samples plated on mycoplasma media (UC-Davis). Bacteriological interpretation and identification was based on the NMC recommendation for bovine milk cultures (Laboratory handbook on bovine mastitis, 1999). Phenotypic characteristic were observed, and Gram stain was used to differentiate *Staphylococcus spp* and *Streptococcus spp*. Coliforms bacteria were distinguished from other Gram negative bacteria using lactose and oxidase reactions. *Staphylococcus* and *Micrococcus* genera were identified on the basis of mannitol and coagulase

tube test. Final bacterial identifications were performed using a miniaturized biochemical system (API Staph Biomérieux SA; Marcy-l'Etoile, France). Only profiles with an identification of at least 80% confidence were used in further analysis at species level. For all other microorganisms, identification was limited to genus.

3.2.4. Definitions

Intramammary infection (IMI) was defined as growth of ≥500 cfu/ml) of identical colonies. Negative culture (NG) was defined as absence of growth. No significant growth (NSG) was defined as growth of <3 identical colonies (<500 cfu/ml). Mixed culture was defined as significant growth of two different types of colonies with ≥5 identical colonies. Contamination was defined as significant growth of $\geq$3 colony types. A ewe was considered to have an IMI if at least one half udder had an IMI infection, and was considered uninfected if both half udders were NG or NSG. In the assessment of IMI, ewes with only one half udder sample available (due to missing or contaminated samples) were excluded, unless the available sample was considered to have an IMI.

3.2.5. Statistical analysis

Logistic regression (PROC LOGISTIC; SAS vers. 9.1) was used to investigate the effect of the treatment on the prevalence of IMI status at ewe level either at lambing or follow up.

Intramammary infection status was the binary (infected, uninfected) response variable. The probability of observing an intramammary infection ($Y_i = 1$) is π_i and the logit of observing the intramammary infection (Y_i) is:

$$\log [\pi_i/(1- \pi_i)] = \eta_i$$

where η_i is the linear predictor of the logistic regression model.

Because π is the probability that $Y_i = 1$, it follows that $1-\pi$ is the probability of $Y = 0$; then, $\pi_i/(1-\pi_i)$ is the odds ratio of the two probabilities.

The effect of treatment, lactation number (parity), number of SCC test in the previous lactation greater than 400,000 cells/ml SCC on IMI status were evaluated using the following model:

$$\log [\pi_i/(1-\pi_i)] = \beta_0 + \beta_1 D_t + \beta_2 L_k + \beta_3 S_l$$

where:

$[\pi_i/(1-\pi_i)]$ is the logit of observing an IMI, β_0 is the intercept, $\beta_1 D_t$ is the effect of dry treatment (t = treated, not treated), $\beta_2 L_k$ is the effect of lactation number (k = young if second and third lactation; old if fourth and greater lactation); $\beta_3 S_l$ is the effect of the number of tests in the previous lactation with SCC ≥ 400,000 cells/ml (<3, ≥3). Separate logistic regression models were run for the outcome IMI at lambing and IMI at follow up. The assessment of IMI was based on the recovery of any pathogen (*Staphylococcus aureus*, Coagulase Negative Staphylococci, *Corynebacterium* spp., *Bacillus* spp., *Enterobacteriacee*, *Streptococcus spp*., other), but only IMI caused by Gram positive bacteria were used in the analysis. Contaminated samples and yeasts were excluded from the analysis and considered separately.

In order to normalize the SCC distribution, the raw DCC was transformed in $\log_{10}$ before the analysis. Its mean was then compared by group of pathogens isolated (PROC ANOVA; SAS vers. 9.1). The groups were defined as: uninfected, major pathogens (*Staphylococcus aureus*, *Enterobacteriacee* and *Streptococcus* spp.), minor pathogens (Coagulase Negative Staphylococci, *Corynebacterium* spp. and *Bacillus* spp.), other and yeast.

3.3. Results

3.3.1. Treatment allocation and study population

Dry treatments were administered to 125 ewes (n = 30 High SCC and n = 95 Low SCC), and 120 ewes were assigned to NT (n = 27 High SCC and 93 Low SCC). Of 245 enrolled ewes, follow-up milk samples were obtained from 214 ewes. Ewes (n=31) were removed from the study because of failure to conceive (n = 14), death (n = 8), or various management reason (n = 9). Treatment group (DT, NT) was not associated with retention in the study (P = 0.96). Groups, treatment assigned and retention are summarized in Table 1. There was no association between assignment to treatment group and retention in the study (P = 0.37 and P = 0.38 at lambing and at follow-up, respectively).

The log_{10}SCC at last test before enrolment was 5.1 for both treatment groups (DT and NT) and, as expected based on the stratified random sampling plan, there was no association (P = 0.78) between assignment to treatment groups (DT, NT) and SCC levels (High, Low). The mean milk production was 1.65 and 1.62 kg for DT and NT, respectively, and no significant difference (P > 0.53) was found based on group allocation. The parity class was no associated with treatment (P = 0.97).

Table 1. Summary of assigned treatment groups and retention

	DryTherapy[a]		No Treatment		
	High[b] SCC	Low[c] SCC	High SCC	Low SCC	Total
Assigned	30	95	27	93	245
Lambing	30	80	24	85	219
Follow-up	29	79	23	83	214

[a]300 mg cephapirin benzathine; [b] ≥ 400,000 cells/ml; [c] < 400,000 cells/ml

3.3.2. Bacteriology

A total of 435 and 426 half udder milk samples were collected at lambing and follow up, respectively (Table 2). Contamination occurred in 28 (6.4%) half udder milk samples collected at lambing and in 3 (0.7%) samples collected at follow up. Uninfected samples accounted for the majority of milk samples (n = 311, 71.0% at lambing; n = 365, 85.7% at follow-up) (Graphic 1 and 2). The prevalence of IMI at lambing was 22.0% (n=96) and 13.6% (n=58). A total of 109 isolates were recovered at lambing and 59 at follow up, respectively. Coagulase Negative Staphylococci (CNS) were the most prevalent pathogens recovered at both sampling time (n = 49; 45.0% at lambing and n = 28; 47.5% at follow-up) (Table 3). Of CNS that were speciated (n = 77) the most prevalent species was *S*. *xylosus* (Table 3).

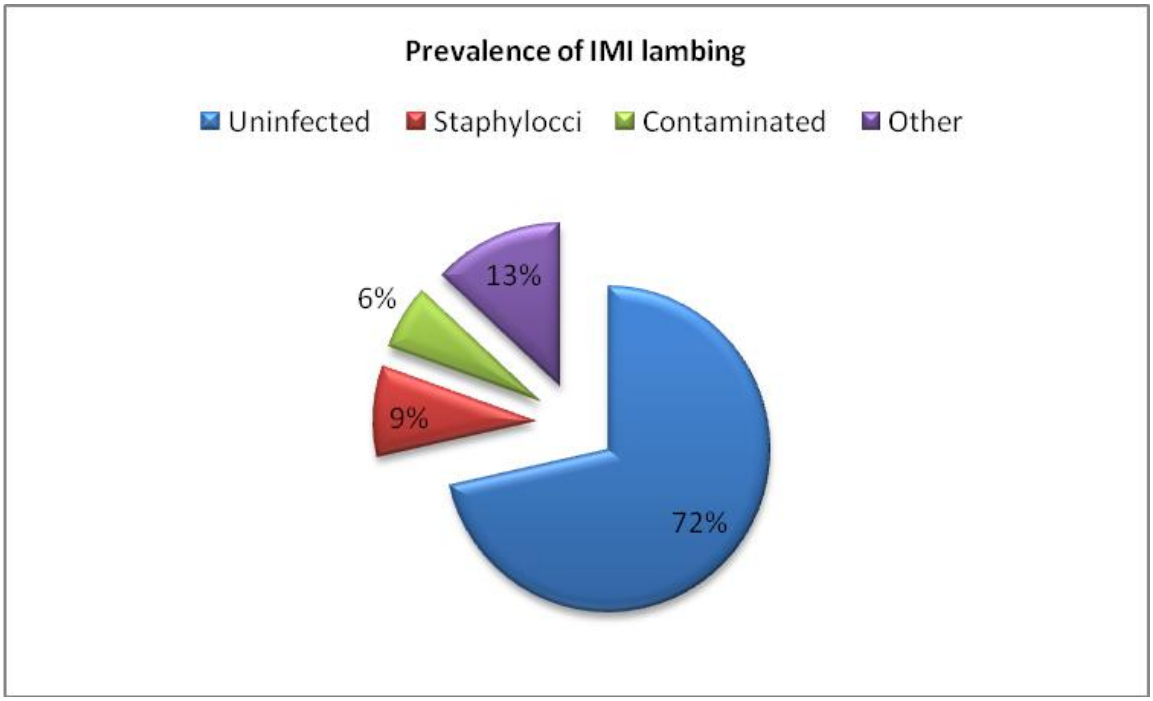
Yeast were detected in milk samples obtained at both lambing (n = 14; 12.8%) and at follow-up (n = 16; 27.1%). Yeast IMI were found only in half udders assigned to the treatment group. The prevalence of *Corynebacterium* spp. and *Bacillus* spp. was greater at lambing (2.7% and 1.6%) than at follow-up (0.5% and 0.7%). *Staphylococcus aureus* was isolated from only the same half udder sample at both lambing and at follow up representing 0.9% and 1.7% of the isolates, respectively.

Table 2. Summary distribution of half udder milk samples collected during the trial.

	Lambing 14-21									
	Treated		No Treated		Total		Treated		No Treated	
	ewes	half udder	ewes	half udder	ewes	half udder	ewes	half udder	ews	half udder
Total enrolled	125	250	120	240	245	490	110	219	109	218
Dead	3	6	2	4	5	10	1	2	2	4
Failed to conceive	8	16	6	12	14	28	-	-	-	-
Culled	4	8	3	6	7	14	1	2	1	2
Missing* samples	-	3	-	-	-	3	-	-	-	1
Retained	110	217	109	218	219	**435**	108	215	106	211

*Blind udder or not collected samples

Graphic 1. Prevalence of IMI at lambing.



Graphic 2. Prevalence of IMI at follow up.

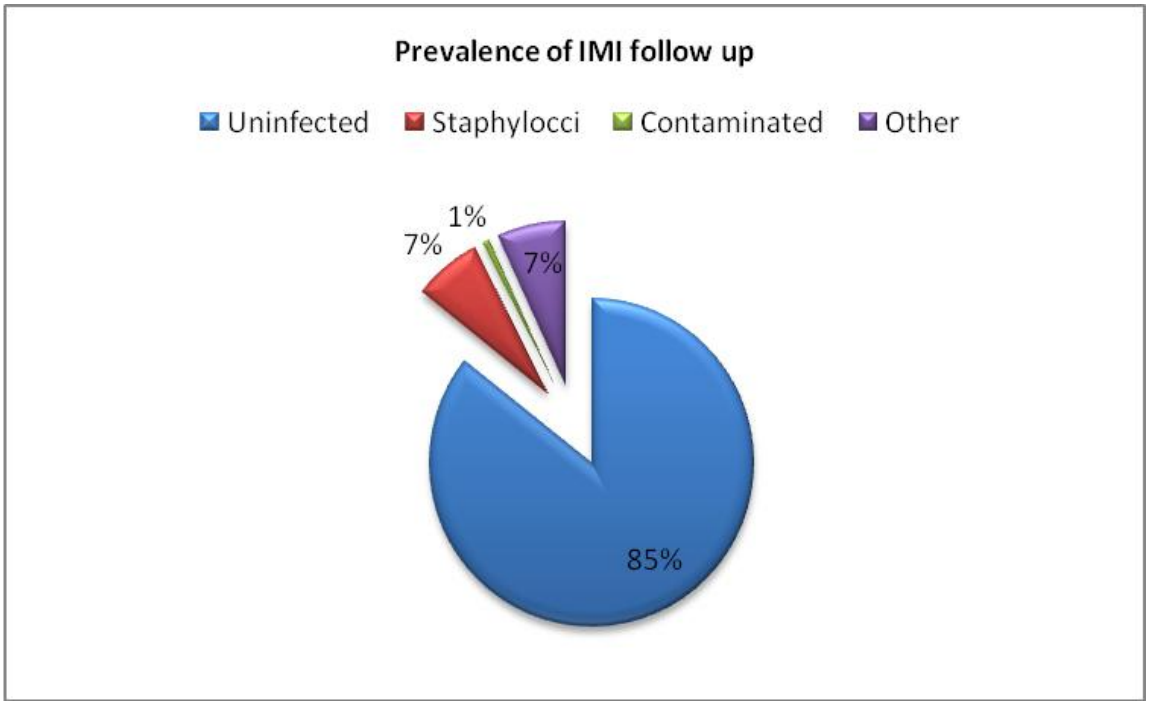


Table 2. Distribution of pathogens recovered from half udder milk samples at lambing and at follow-up.

Species	Lambing		Follow-up[c]	
	n	%	n	%
CNS[a]	49	45.0	28	47.5
Corynebacterium spp	12	11.0	2	3.4
Yeast	14	12.8	16	27.1
Other	12	11.0	4	6.8
Enterobacteriacee	8	7.3	2	3.4
Bacillus spp	7	6.4	3	5.1
Streptococcus spp	6	5.5	3	5.1
Staph.aureus	1	0.9	1	1.7
Total	109	100.0	59	100.0

Table 3. Prevalence of CNS IMI by species at lambing and follow-up.

Species	Lambing		Follow-up	
	n	%	n	%
Unidentified	3	6.1	2	7.1
S. xylosus	13	26.5	5	17.9
S. chromogenes	10	20.4	2	7.1
S. epidermidis	10	20.4	2	7.1
S. auricularis	4	8.2	7	25.0
S. simulans	4	8.2	2	7.1
Micrococcus spp.	1	2.0	3	10.7
S. cohnii	1	2.0	2	7.1
S. lentus	1	2.0	-	-
S. capitis	1	2.0	-	-
S. hominis	1	2.0	-	-
S. caprae	-		1	3.6
S. warneri	-		2	7.1
Total	49	100.0	28	100.0

3.3.3. Intramammary infection

The logistic regression was performed on data obtained from 181 ewes at lambing and from 194 ewes at follow-up, respectively. The probability of IMI was not associated (P> 0.18) with DT at lambing or at follow-up (Table 4). The effect of lactation number on IMI was significant at lambing (P = 0.03) but not significant at follow-up (P = 0.90). Ewes with 3 or more monthly test ≥ 400,000 cells/ml in the previous lactation were 5.6 times more likely to be infected at lambing and 7.5 times more likely to be infected at follow up (P< 0.001)(Table 4).

Table 4. Estimates of IMI probabilities at lambing and at follow-up.

		Lambing		Follow-up	
	levels	O.R[a].	P value	O.R.	P value
Dry Treatment	Not treated	1.60	0.18	1.29	0.53
	Treated	-		-	
Number of test[b]	>3	5.63	<.0001	7.52	<.0001
	≤3	-		-	
Lactation number	2 and3	2.07	0.03	0.96	0.90
	>3	-		-	

[a]Odds Ratio Estimates. [b]Number of monthly SCC test in the previous lactation ≥400,000 cells/ml.

3.3.4. DeLaval Somatic Cell Count (DCC)

The DCC was evaluated on 426 half udder milk samples collected at follow up (14-21 day after lambing). The mean Log10SCC was 4.70 and was significantly different (P < .001) among pathogens. Greater SCC was observed for minor pathogens (5.8) and differed among

uninfected, yeast and other. As expected the least count (4.57) was found in the uninfected group (Table 5).

Table 5. Log SCC by group of pathogens at follow-up

	n obs	mean	SD	SE
Major	6	5.64 [a b]	0.94	0.38
Minor	32	5.79 [a]	0.73	0.13
Other	4	4.72 [c d]	0.58	0.29
Uninfected	365	4.57 [d]	0.41	0.22
Yeast	16	5.30 [b c]	0.35	0.09

Means that have the same letter are not significantly different

3.4. Discussion

In agreement with previous studies, CNS were the most prevalent pathogens recovered (Fthenakis, 1994; Burriel, 1997; Lafi et al., 1998; Ariznabarreta et al., 2002; Gonzalo et al., 2002; Bergonier and Berthelot, 2003; Hariharan et al., 2004). Among CNS, *Staphylococcus xylosus* (26.5%), *Staphylococcus auricularis* (20.4%), *Staphylococcus chromogenes* (8.2%) and *Staphylococcus epidermidis* (8.2%) were the predominant species recovered. Speciation of CNS with phenotypic tests such as API Staph ID 32, is dependent upon laboratory methods and in research genotypic methods for species identification should be preferred over phenotypic methods (Sampimon et al., 2008). However, at diagnostic level genotypic method are not yet implemented as routine test. Somatic cell count of uninfected udder was lower when compared with other studies (Green, 1984; Maisi et al., 1987: Gonzalo et al., 1994b; González-Rodríguez et al., 1995 ;). Although CNS in this study elicited SCC lower than reported in literature (Pengov, 2001) the response to the intramammary infection was greater than the

response observed in cows. In dairy cows, CNS are generally considered minor pathogens (Dohoo and Meek, 1982; Bergonier et al., 1996) but CNS induce great SCC responses in dairy ewes (Pengov, 2001) and these pathogens are the primary cause of subclinical mastitis in dairy sheep. A greater SCC in IMI caused by minor pathogens was observed in this study, supporting other author's findings (Pengov, 2001) that CNS in dairy ewes cannot be considered as minor pathogens, since they elicit high SCC. Moreover CNS IMI are more likely to occur in ewes with a history of high SCC (odds ratio ranging between 5.63 and 7.52) confirming the chronic course of these pathogens.

The greater prevalence of contaminated samples from lambing to follow-up may be explained by the fact that the samples were not collected by the same personnel. Of the 30 yeasts isolated, all cases were isolated from half udder assigned to the treatment group. Little is known about mycotic mastitis in small ruminants, but an association between incorrect administration (such as contaminated drugs and syringes) of antibiotic at drying-off was suggested (Las Heras et al., 2000). Of ewes included in the study, 30 cases of intramammary yeast infection were identified and all cases occurred in the treatment group. Mycotic mastitis is usually associated with intramammary infusion with contaminated syringes (Paine, 1952; Loftsgard and Lindquist, 1960; Mantovani et al., 1970; Farnsworth and Sorensen, 1972; Thompson et al., 1978; Richard et al., 1980; Kirk and Bartlett, 1986; Krukowski et al., 2000). Few cases of mycotic mastitis by *Aspergillus fumigatus* have been reported in small ruminants (Berthelot and Bergonier, 1993; Jensen et al., 1996; Pérez et al., 1998). An association between mammary aspergillosis and incorrect administration of antibiotic at drying off has been previously observed in dairy ewes (Las Heras et al., 2000). In this study, all cases of yeast mastitis were isolated from milk samples obtained from half udders assigned to the treatment group, indicating farm personnel should be extremely diligent in using strictly hygienic

conditions during administration of intramammary DT. Although this study did not show any significant effect of dry treatment on the prevalence of IMI, it declined with time post partum regardless the dry treatment. Similar trend has been shown in other studies (Hueston et al., 1986; Kirk et al., 1996; McDougall et al., 2002). In this study, ewes with ≥ 3 monthly test with SCC ≥ 400,000 cells/ml were up to 7.5 times more likely to have mastitis infections in the period postpartum when compared to ewes with <3 test days with high SCC. Our results highlight the importance of SCC as predictor of IMI and of using SCC test day reports as a mastitis management tool.

3.5. Conclusion

The high prevalence of yeast infections demonstrates the importance of proper antibiotic administration procedures. To avoid potential yeast infections, dry therapy should be performed under strict hygienic conditions, using sterile products and equipment and following proper sanitation procedures. Results of different studies agree with the effectiveness of dry off therapy for prevention and treatment of mastitis (Hueston et al., 1989; Gonzalo et al., 2004; Chaffer et al., 2003). Our study was not able to show any significant effect of reducing the IMI. These results are probably due to the elevated number of yeast IMI induced with the treatment. Though, we were still able to confirm the importance of SCC as a predictor of IMI and its possible use as a treatment decision criterion. Ewes with a history of high somatic cell count (i.e. more than 3 test during the lactation ≥ 400,000 cells/ml) are more likely to have IMI in the post parturition period. As a general rule before the dry treatment is recommended on a regular basis, other factors, such as cost benefit ratio, target pathogens (i.e. CNS), implementation of hygienic milking and management strategies must be considered.

Chapter 4
Impact of teat sanitation on somatic cell count in dairy ewes

4.1. Introduction

Teat disinfection is a practice meant to decrease the risk of intramammary infection by reducing bacteria population on teat skin (Bramley et al., 1996). This is a well established practice in dairy cattle (Philpot and Pankey, 1975; Natzke, 1977; Philpot, 1979; Farnsworth, 1980; Pankey et al., 1984; Galton et al. 1986; Rasmussen et al. 1991; Oliver et al. 1993; Nickerson, 2001; Magnusson et al. 2006). Teat disinfection may be conducted just before milking and is termed pre milking teat disinfection (predipping), or immediately after milking and is termed post milking teat disinfection (postdipping). Postdipping is aimed at destroying bacteria on the teat ends immediately after removal of the teatcups, and is effective in the control of contagious mastitis by minimizing their further spread into the gland. Predipping is intended to combat environmental pathogens by reducing microbial population on teat skin before milking. Teat disinfection with low iodine concentration formulations proved to reduce the incidence of IMI in dairy cattle (Bushnell, 1984; Galton et al., 1984; Galton et al., 1988; Pankey and Galton, 1989; Blowey and Collis, 1992; Langridge, 1992; Oliver et al., 1993; Skrzypek et al., 2004). There are several arguments regarding the use of teat sanitation as a milking routine. The dipping with iodine products increases the risk of iodine residues in milk (Galton et al., 1986b). Contamination may be by absorption through the teat skin or aspiration of residual iodine left on the teat surface by the preparation process (Conrad and Hemken, 1978). Galton et al. (1986) showed that, provided the teat are adequately wiped afterward, premilking dipping with a 0.1% iodophor dip had no significant effect on milk iodine levels, but that 0.5% iodophor preparation led to increases in milk iodine. Other study (Aumont, 1987) concluded that post milking teat disinfection with 0.5% iodophor produced only a small increase in milk iodine. Accurate drying of teats with paper towels after predipping is needed to reduce iodine residue in milk (Galton et al., 1984; Galton et al. 1986; Rasmussen et al., 1991;

Ruegg, 2004), and is a recommended practice (National Mastitis Council, 2004). Although there is large literature of teat sanitation in dairy cows, little and controversial information is available for dairy ewes. Teat dipping was very effective in preventing new IMI (Contrearas et al., 2007) while it was ineffective in to restore udder health in sheep with subclinical mastitis (Klinglmair, 2005). The objective of this study was to assess the impact of premilking teat sanitation on somatic cell count trough an entire lactation in dairy sheep.

4.2. Materials and methods

The study was conducted at the University of Wisconsin-Madison dairy sheep research facility. The flock consists of East Friesian, Lacaune, East Friesian-Lacaune crossbreeds and crossbreds with meat ewes. Lambing occurred in late January through March 2008, and ewes were milked until late fall (late September or early October).

4.2.1. Allocation to groups

After 14-21 days in lactation, eligible ewes were randomly assigned to a premilking sanitation treatment. Multiparous ewes were previously enrolled in the antibiotic dry off treatment study, so ewes were randomly assigned to teat sanitation (predipping and no predipping) within each dry treated group (dry treated and no dry treated). Primiparous ewes were randomly assigned either to predipping or no predipping. The effectiveness of the allocation process was evaluated using chi square analysis (PROC FREQ vers. 9.1). Ewes assigned to teat sanitation (pre-dipping) had their teats immersed in 0.5% iodine before unit attachment. The predip was allowed to have a contact time of at least 30 seconds before it was dried off using disposable individual paper towels. Ewes assigned to the control group received no premilking teat sanitation. Sheep were clearly marked with leg tags so that milking personnel could easily

determine which group the animals belonged to. All ewes received postmilking teat dip throughout the entire lactation period. Test day reports, with milk production and SCC data, were obtained from Dairy Herd Improvement Agency (DHIA) monthly records for the entire lactation. Bulk milk samples were checked for antibiotic residues at least twice a week.

4.2.2. Statistical analysis

Monthly somatic cell count

Using monthly test day report data, an independent group t-test (PROC TTEST_IND.SAS vers. 9.1) was used to compare mean somatic cell count between sanitation group (predipping and no predipping) for the entire duration of the lactation.

Somatic cell count level and sanitation

The association of teat sanitation with current SCC was tested with a chi square analysis (PROC FREQ; SAS vers. 9.1). In the contingency table the rows were defined as pre milking sanitation (PD) and no sanitation (ND), the columns were defined as ewes with at least one SCC test in the current lactation greater or equal to 400,000 cells/ml and ewes with no SCC test in the current lactation greater or equal to 400,000 cells/ml. Only ewes with at least 7 complete test day reports were included in the analysis. To take into account the SCC history ewes were blocked on the basis of their test day SCC in the previous lactation (only multiparous were included). The blocks were ewes with at least one monthly test in the previous lactation greater or equal to 400,000 cells/ml (High SCC) or ewes with all the monthly SCC test in the previous lactation less than 400,000 cells/ml (Low SCC).

4.3. Results

4.3.1. Treatment allocation and study population

A total of 216 multiparous ewes and 61 primiparous ewes were enrolled in the premilking teat sanitation study. After the randomization the groups were as follow: 1) dry ewe treatment + predip (n= 65); 2) dry ewe treatment – no predip (n=42); 3) no dry treatment + predip (n= 64); 4) no dry treatment – no predip (n=45). Primiparous ewes were randomly assigned either to premilking teat sanitation (n= 31) or no premilking teat sanitation (n= 30) (Table 1). The 4 treatment groups were homogeneous.

4.3.2. Monthly somatic cell count

Somatic cell count was $\log_{10}$ transformed before the analysis in order to approximate a normal distribution. For multiparous ewes up to 9 monthly tests were available, while for primiparous ewes, which lamb later, up to 7 monthly reports were available. In multiparous ewes the overall SCC ranged between 4.59 and 4.96, mean SCC by group is reported in Table 2. In primiparous ewes the mean SCC ranged between 4.39 and 5.11, mean SCC by group is reported in Table 3. The independent group t-test showed no significant difference ($P>0.05$) between the sanitation groups across the lactation for both first lactation and multiparous ewes (Graph 1 and 2).

4.3.3. Somatic cell count level and sanitation

Only multiparous ewes with at least 7 monthly tests in the current lactation and with complete SCC history of the previous lactation were included in the statistical analysis. Over 209 eligible ewes at the beginning of the lactation (PD= 105, ND= 104) by the end of the campaign, complete monthly data reports were obtained for 180 ewes. After blocking on the basis of the SCC in the previous lactation (ewes with at least one SCC test day greater or equal to 400, 000

cells/ml), the groups were: 88 ewes that received PD (n = 47 High SCC and n = 41 Low SCC), and 92 ewes received ND (n = 48 High SCC and n = 44 Low SCC). Treatment group (PD, ND) was not associated with retention in the study (P = 0.55) (Table 4). At the end of the current lactation the ewes with at least one test above 400,000 cells/ml were 39 and 44 in the PD and ND group, respectively. The number of ewes with no test in the current lactation above 400,000 cells/ml was 49 in the PD group and 48 in the ND one. Overall no association was found between the treatment (PD) and having at least one monthly SCC test in the current lactation greater or equal to 400,000 cells/ml (P = 0.75) (Table 5). Blocking the ewes in two groups on the basis of their SCC history, those with at least one monthly SCC ≥400,000 cells/ml (previous High), and those with no SCC ≥400,000 cells/ml (previous Low) in the previous lactation, did not show any significant effect on the current SCC. The number of ewes with at least one SCC test ≥400,000 cells/ml was not associated with treatment (PD, ND) in both SCC block, P = 0.97 and p = 0.27, respectively. To account for the effect of age, the ewes were stratified by parity. The groups were defined as young (2^{nd} and 3^{rd} parity) and old (4^{th} and older). In ewes with a history of high SCC and in the old parity group the teat dipping was associated with having in the current lactation at least one test SCC ≥400,000 cells/ml (0.05) (Table 5).

4.4. Discussion

The objective of premilking teat sanitation is to reduce bacterial colonization of teat skin. The use of predipping in dairy ewes should be justified by the consideration that the prevalence by contagious pathogens (*S. aureus* and *Streptococcus agalactiae*) is low. Premilking teat hygiene has been demonstrated to effectively reduce bacterial counts of teat skin of dairy cows and is recommended as an effective method to reduce exposure to potential environmental mastitis

pathogens (Pankey and Dreschler, 1993; Galton et al., 1988). In the present study no overall significant effect of sanitation was found on the SCC (P>0.05). The effectiveness of teat sanitation in dairy small ruminants has been previously evaluated. In one study (Contrearas et al., 2007) teat sanitation was very effective in reducing new IMI although the flock had a high prevalence of infected animals. Klinglmair (2005) reported no significant effect of sanitation in his trial. However, both studies evaluated the use of post milking sanitizer. The results could be justified by the fact that the mean SCC in the flock was low, making it difficult to detect some effect. The primary source of environmental pathogens includes bedding, manure, and soil, typical of factory farming. The raising system of dairy ewes may reduce the exposure of teat to environmental pathogens. In dairy ewes CNS are the most frequently recovered pathogens (Fthenakis, 1994; Burriel, 1997; Lafi et al., 1998; Ariznabarreta et al., 2002; Gonzalo et al., 2002; Hariharan et al., 2004), and although they are normally present on teat surface and they elicit high SCC levels, no significant effect of sanitation on SCC levels was found in this study. Previous research in dairy cattle has demonstrated that predipping is not always cost effective when CNS are the predominant mastitis pathogens (Ruegg and Dohoo, 1997). Teat sanitation was effective in ewes with increased risk (history of high somatic cell count and older parity group), indicating an eventual application, but further research is needed to evaluate the effect of premilking teat sanitation on reduction of bacterial count and on the incidence of intramammary infections.

4.5. Conclusion

Pre milking sanitation is a well established routine in dairy cows. Our study did not support the use of sanitation with a 0.5% iodine solution before milking dairy sheep. A potential application of teat sanitation as a premilking routine might be advisable in flocks with high

somatic cell count level. The feasibility of this practice should also take into account the reticence of dairy farmers to implement such procedure, considering the elevated number of head to treat and the negative effect on parlour throughput.

Table 1. Premilking teat sanitation (predipping) assignment groups.

	Dry treated			Not Dry treated			
Ewes	Predipped	Not predipped	Total	Predipped	Not predipped	Total	Total
	n	n		N	n		n
Multiparous	65	42	107	64	45	109	216
Primiparous	.	.	.	31	30	61	61

Table 2. Multiparous mean Log_{10}SCC during lactation by sanitation group.

Groups	**Test1**		**Test2**		**Test3**		**Test4**		**Test5**		**Test6**		**Test7**		**Test8**		**Test9**	
	n	**μ**	**n**	**μ**	**n**	**μ**	**n**	**μ**	**n**	**μ**	**n**	**μ**	**n**	**μ**	**n**	**μ**	**n**	**μ**
Predip	129	4,77	124	4,71	122	4,95	120	4,93	120	4,69	116	4,56	108	4,68	95	4,67	30	4,56
No Treated	65	4,70	63	4,75	62	5,01	59	4,93	59	4,66	58	4,41	55	4,65	46	4,59	15	4,46
Treated	64	4,85	61	4,68	60	4,90	61	4,93	61	4,72	58	4,71	53	4,71	49	4,74	15	4,67
No predip	87	4,75	82	4,87	77	4,98	74	4,93	74	4,67	71	4,90	68	4,71	53	4,47	15	4,63
No Treated	42	4,70	39	4,86	39	5,06	36	4,96	36	4,57	35	5,09	34	4,78	26	4,50	7	4,97
Treated	45	4,79	43	4,89	38	4,91	38	4,90	38	4,76	36	4,71	34	4,63	27	4,45	8	4,33
Total	216	4,76	206	4,78	199	4,96	194	4,93	194	4,68	187	4,69	176	4,69	148	4,60	45	4,59

Table 3. Primiparous mean Log_{10}SCC during lactation by sanitation group.

Groups	Test1		Test2		Test3		Test4		Test5		Test6		Test7	
	n	µ	n	µ	n	µ	n	µ	n	µ	n	µ	n	µ
Predip	30	5,12	30	5,11	30	4,85	30	4,30	27	4,64	24	4,47	11	4,45
No predip	31	5,10	30	5,03	30	5,00	28	4,50	26	4,59	20	4,53	9	4,86
Total	61	5,11	60	5,07	60	4,92	58	4,39	53	4,61	44	4,50	20	4,64

Table 4. Effect of sanitation on current somatic cell count: summary of **e**wes enrolled and completing the study .

	Pre Dipped[a]			Not Dipped			
	previous High[b]	previous Low[c]	Total	previous High[b]	previous Low[c]	Total	Total
	n	n		N	n		n
Assigned	55	50	105	54	50	104	209
Retained[d]	41	47	88	48	44	92	180

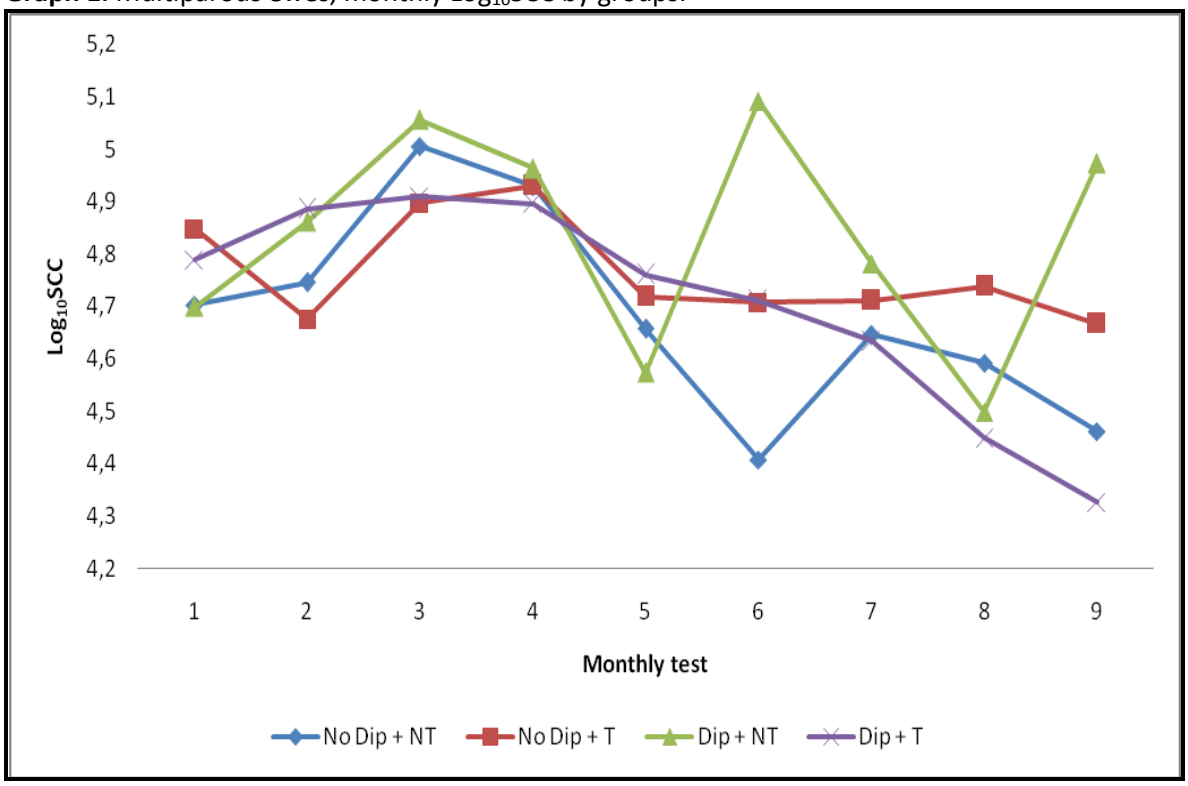
[a] Pre milking sanitation with 1% iodine solution; [b] Ewes with at least one monthly SCC test day greater or equal to 400,000 cells/ml in the previous lactation; [c] Ewes with no monthly SCC test day greater or equal to 400,000 cells/ml in the previous lactation. [d] Ewes with complete report at the end of the lactation.

Table 5. Test of association between teat sanitation and current SCC.

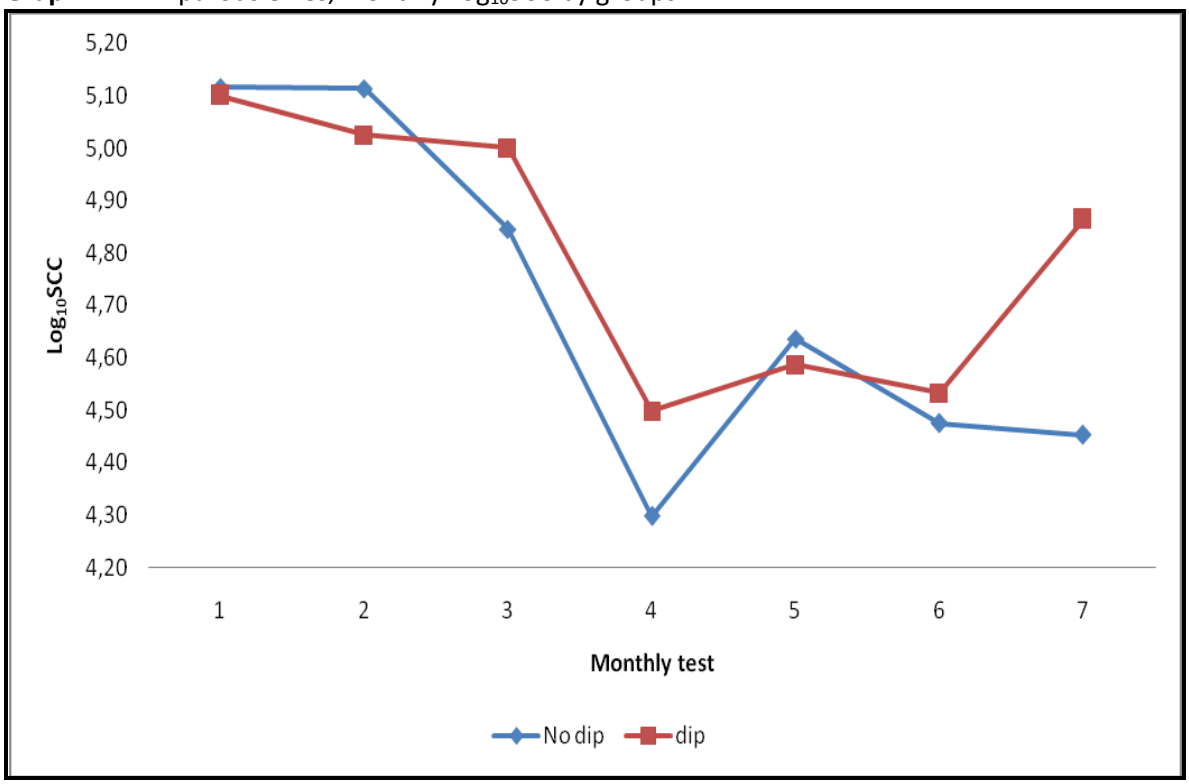
	Pre Dipped[a]			Not Dipped			
	current High[b]	current Low[c]	Total	current High[b]	current Low[c]	Total	
	n	n	n	n	n		p-value
Overall	39	49	88	44	48	92	0.75
Previous Low[d]	20	27	47	15	29	44	0.54
Previous High[e]	19	22	41	29	19	48	0.27
- young[f]	16	7	23	14	6	20	0.97
- old[g]	5	13	18	16	12	28	0.05

[a] Pre milking sanitation with 1% iodine solution; [b] Ewes with at least one monthly SCC test day greater or equal to 400,000 cells/ml in the current lactation; [c] Ewes with no monthly SCC test day greater or equal to 400,000 cells/ml in the current lactation. [d] Ewes with at least one monthly SCC test day greater or equal to 400,000 cells/ml in the previous lactation; [e] Ewes with no monthly SCC test day greater or equal to 400,000 cells/ml in the previous lactation; [f] Ewes in the 2nd and 3rd parity; [g] Ewes 4th parity and older.

Graph 1. Multiparous ewes, monthly Log_{10}SCC by groups.



Graph 2. Primiparous ewes, monthly Log_{10}SCC by groups.



Chapter 5
Combined effect of antibiotic dry treatment and teat sanitation on somatic cell count in dairy ewes

5.1. Introduction

The hygienic quality of milk is strictly related to the health status of a flock. As a consequence dairy farmers and sheep-breeding organisation put in place different mastitis control programs. The goals of those programs are to prevent new infections and to eliminate the existing infections. Among the possible strategies adopted there is teat dipping with disinfectant solutions and antibiotic dry off treatment. The effect of these measures on milk hygiene has been discussed in chapter 3 and 4. The objective of this chapter was to assess the combined impact of antibiotic dry off treatment and premilking teat sanitation on somatic cell count.

5.2. Materials and methods

The study site, the study population, the assignment of treatment (dry therapy and teat sanitation) are described in chapter 3 and 4. A brief description follows. At the end of the lactation (October 2007), 245 milking ewes were blocked on the basis of the current test day SCC, and randomly allocated to either antibiotic intramammary treatment (DT) or no treatment (NT). The blocks were ewes with the last monthly SCC greater or equal to 400,000 cells/ml (High SCC) or ewes with the last monthly SCC less than 400,000 cells/ml (Low SCC). During the last milking session, ewes assigned to DT received intramammary infusion of a commercially available dairy cow dry product that contained 300 mg cephapirin benzathine. Two days and 14-21 days post lambing (follow-up) half udder milk samples were aseptically collected for bacteriology (Chapter 3). After the collection of the follow-up sample ewes were randomly assigned within treatment group to a premilking sanitation treatment (Table1). Ewes assigned to teat sanitation (PD) had their teats immersed in 0.5% iodine before unit attachment. Ewes assigned to the control group received no premilking sanitation. Monthly milk production and SCC data were obtained from the Dairy Herd Improvement Agency (DHIA)

records for the entire lactation. The effect of DT and pre-milking sanitation on mean SCC in the subsequent lactation was evaluated using a mixed model (PROC MIX SAS; vers. 9.1). To take into account the effect of treatment through the whole lactation a repeated measure with autoregressive correlation was used. The response variable was transformed to Log_{10} values to approximate a normal distribution.

The model used was:

$$Ypts = \mu + Dt + Sp + Ts + Dt*Ts + e$$

Where (Y) was the response variable log_{10} SCC, μ is the overall mean, D was the fixed effect of treatment the treatment (t = dry treated, not treated), S was the effect of sanitation (p = predipped, not predipped), T was the effect of the number of test in the previous lactation ≥ 400,000 SCC/ml (s = <3, ≥3), Dt*Ts was the interaction between dry treatment and SCC test, *e* was the error.

Only ewes with complete available data for up to 7 monthly tests during the lactation were included in the analysis. Ewes with yeast IMI were excluded from statistical analysis because the infection was a consequence of the treatment (Chapter 3).

Table 1. Summary of assigned treatment groups

Sanitation	**No Treated**			**Dry Treated**[a]			
	High SCC	Low SCC	Total	High SCC	Low SCC	Total	Gran total
No dipping	12	41	53	16	38	54	107
Dipping	13	40	53	14	40	54	107
Total	25	81	106	30	78	108	214

[a]300 mg cephapirin benzathine; [b] ≥ 400,000 cells/ml; [c] < 400,000 cells/ml

5.3. Results

The combined effects of DT and pre-dipping on monthly log_{10}SCC were evaluated using 1247 test day observations over 7 months. Monthly data reports were available from a minimum of 179 to a maximum of 214 ewes. The average flock log_{10} SCC through the 7 months of lactation was 4.8. The point estimates of the different predictors used in the regression are reported in Table 2. The effect of parity was tested showing no significant impact ($p = 0.67$) and then removed from the final model. The log_{10} SCC was greater in the not treated group (5.0) as compared to the dry treated (4.8) indicating the effectiveness of the antibiotic dry treatment in lowering the SCC. The greatest effect was found for ewes with greater SCC in the previous lactation ($p < .0001$). Ewes with 3 or more SCC test ≥ 400,000 cells/ml in the previous lactation had a mean log_{10} SCC greater than the ewes with less than 3 test over the threshold of 400,000 cells/ml (5.2 vs. 4.6). The effect of treatment and SCC test was evident also when an interaction term was introduced, although not significant ($p = 0.06$). The mean SCC was lower for ewes with less than 3 test ≥ 400,000 cells/ml in both dry treated and not treated group (Table 5). The sanitation before milking had no significant effect on the overall mean log_{10}SCC. The mean log_{10} SCC estimates were 4.9 and 4.8 for the pre-dipped and non pre-dipped group, respectively.

Table 2. Outcome of regression equations on SCC.

Effect		Estimate	p -value
Dry Treatment			0.01
	DT	4.82	
	NT	5.00	
Sanitation			0.15
	Yes	4.96	
	No	4.87	
SCC test			<.0001
	≥3	5.19	
	<3	4.64	
Treatment*SCCtest			0.06
	NT, <3	4.66	
	NT, ≥3	5.34	
	DT, <3	4.61	
	DT, ≥3	5.03	

Dry treatment = 300 mg cephapirin benzathine; Sanitation = predipping with 0.5% iodine; SCC test = number of test in the previous lactation with SCC ≥400,000 cells/ml; Treatment*SCCtest = interaction between dry treatment and SCC test.

5.4. Discussion

The use of intramammary antibiotic dry treatment is a mastitis control strategy that has been proven be effective in reducing the prevalence of IMI in dairy ewes (De Santis et al., 2001; Chaffer et al., 2003; Gonzalo et al., 2004). Unfortunately at farm level bacteriological analysis is not always promptly available to select animals to be treated. In this study an alternative approach was used to identify ewes eligible for antibiotic intramammary treatment at dry off. Somatic cell count records were used to select ewes with a history of high SCC. Although the dry treatment did not show any significant effect on the prevalence of IMI at lambing and at follow-up (Chapter 3), the effect was evident on the SCC. The little effect on IMI might be explained with the low prevalence of IMI in the flock (< 14% at follow up). As expected the SCC history was the most important predictor and had a great impact on the current SCC,

demonstrating the feasibility of using it as an important tool in managing subclinical mastitis. Having more than 3 tests during the lactation over 400,000 cells/ml is an indication of ewes eligible to be dry treated. Moreover, our study demonstrates that ewes that tend to have high SCC during the lactation are more likely to have high SCC in the next lactation, despite the antibiotic treatment at dry off. Thus, the antibiotic dry treatment is advisable in flocks where individual milk samples culture has been performed and characterized by a high prevalence of intramammary infection. In all other circumstances when dealing with recurrent high SCC ewes, culling should be considered.

Discussion

Although sheep dairying is present all over the world, sheep milk production has a well established tradition in Southern and Eastern Europe, in the Middle East and in North Africa (Berger et al., 2004; Pirisi et al., 2007) where sheep milk is mainly used for cheese making. In the USA it was unheard until about 25 years ago, and although is growing rapidly, is still limited (Berger et al., 2004; Thomas and Haenlein, 2004). The increase of international trade of foodstuffs makes even more necessary to ensure the safety of the products placed on the market in order to pursuit a high level of protection of public health. It concerns food business operators, official control and consumers. The quality of milk in the primary production is essential in order to prevent the risk of food-borne diseases in the dairy products chain. With the term milk quality is meant its composition (butterfat and protein) and its hygienic quality (bacterial count and somatic cell count). Milk is sterile when secreted from an uninfected udder; therefore udder health is an essential prerequisite in order to produce hygienic milk. Pathogenic microorganism can be shed in the milk of an infected udder representing a potential hazard to consumer's health. When it comes of milk quality different criteria are applied in different countries. In the EU a limit of 400,000 cells/ml is set for cow milk, but there is no actually a legal limit for SCC of small ruminants. In the US the Food and Drug Administration established a limit of 750,000 cells/ml for cows and 1,000,000 cells/ml for goats and sheep. The limit for bacterial count of raw milk is set a 1,500,000 cfu/ml in the EU and 100,000 cfu/ml in the USA (Regulation EC n. 853/2004, U.S. Grade "A" Pasteurized Milk Ordinance). Should be pointed how in the United States the limits established for cow milk are, with little differences, applied to small ruminants (Haenlein, 1993). Differences exist in the process of milk secretion between cows and small ruminants (Paape et al., 2001), making it discriminatory against sheep and goats the application of such limits (J. Boyazoglu and Morand-Fehr, 2001). Separate standard should be set for small ruminants in order to be achievable for dairy producer to produce milk consistent with the legal limits. With the

introduction in some countries of quality payment systems based on SCC, although this are not a legal issue, farmers are strongly encouraged to maintain udders free from infection (e.g. mastitis). The implementation of comprehensive mastitis control programs is fundamental. A complete control program should include indication for: a) correct diagnosis of mastitis; b) treatment; c) prevention. Among the different strategies to detect mastitis the enumeration of the somatic cell count is one of the most effective tools available for dairy producers, especially when in subclinical form. The SCC is not a public concern itself, in fact they are normally present in the milk, but their count (SCC) is an indicator of udder health and is often used as a predictor of intramammary infection (González-Rodríguez et al., 1995; Barillet et al., 2001; Gonzalo et al., 2002). The current reference method for enumeration of somatic cells recommended by the ISO 13366-1/IDF 148-1 (2008) is the direct microscopic somatic cell count (DMSCC). Automated somatic cell counter such as the Fossomatic^{TM}, have been well standardized for bovine and ewe milk and it compares favorably with the reference method (Heald et al., 1977; Schmidt-Madsen, 1979; Miller et al., 1986; Barcina, et al., 1987; Bertrand, 1996; Barkema et al., 1997; Gonzalo et al., 2003, 2004), but on a practical basis animal side test are to be implemented on farm to be effective management tools. A practical, economic and cheap method is needed at farm level to be an effective tool to control milk quality. The CMT fulfil these requirement, but lacks in objectivity, and though is a useful system in mastitis control, it cannot be used for counting the correct SCC. A more accurate somatic cell count enumeration is needed, especially when somatic cell is used as a criterion in a quality payment system. On farm automated somatic cell counters, such as the *DeLaval Cell Counter* (DeLaval International AB, Tumba, Sweden) have been proposed in dairy cows (DeLaval, 2005). Some differences exist between cow and sheep milk (higher total solids content and higher content of cytoplasmic particles) which calls for specifics operative condition in the use of automated cell counter in sheep milk (Gonzalo et al., 2006 and 2008). An acceptable overall accuracy of

the DCC has been proven for Churra and Assaf breeds when allowing 1 or 2 minutes of "soaking time" (staying of the milk inside the cassette before the reading) (Gonzalo et al., 2008). Chapter 2 was aimed to compare the correlation between DCC and FSCC methods on ovine milk in a sheep flock in the USA. The results of our study are in agreement with previous finding and demonstrate that the DCC can be an efficient and accurate method for enumeration of somatic cells and check for udder health in field conditions, also in ovine milk of Lacune and Eastern Friesian breeds reared in the USA. The measuring range of the DCC (10,000 to 4,000,000 cells/m) is narrower than other methods, however, considering that most decision are based on SCC values within this range, the use of a portable SCC such as the DeLaval Cell counter, is accurate enough to be used in SCC monitoring and control programs. The practical impact of this study reflects on mastitis management strategies. The availability of an automated and rapid system for on farm monitoring of BTSCC and iSCC is a fundamental practice in a mastitis control program. It is even more important in dairy sheep farming, where most of the mastitis occurs in the subclinical form (Lafi et al., 1998; Las Heras et al., 1999; Albenzio et al., 2002; Bergonier and Berthelot, 2003; Contreras et al., 2003), and hence can only be detected with laboratory procedures. Moreover, in countries where quality payment system are applied, BTSCC is one of the parameters considered when determining premiums or penalties on milk price, and as a consequence the somatic cell count has a great impact on the economy of the farmer and of the dairy industry (Dubeuf and Le Jaouen, 2005; Pirisi et al, 2007). *S. aureus* has been reported as the most recovered pathogen from dairy ewes with clinical mastitis (Al-Samarrae et al., 1985; Kirk et al., 1996; Lafi et al., 1998; Ariznabarreta et al., 2002;) while coagulase-negative staphylococci (CNS) are reported to be the most common pathogens recovered from cases of subclinical mastitis of dairy ewes (Fthenakis, 1994; Gonzalez-Rodriguez et al., 1995; Burriel, 1997; Lafi et al., 1998; Las Heras et al., 1999; Pengov, 2001; Leitner et al., 2001; Ariznabarreta et al., 2002; Gonzalo et al., 2002; Hariharan et al.,

2004) and they elicit high SCC (Deinhofer, 1993; Pengov, 2001; Gonzalo et al., 2000). Controlling SCC of raw milk is an important aspect of hygienic milk production and as a consequence of food safety. In our study (Chapter 3) CNS were the most prevalent pathogens recovered, accounting for 45% of the infections. Among CNS, *Staphylococcus xylosus*, *Staphylococcus auricularis*, *Staphylococcus chromogenes* and *Staphylococcus epidermidis* were the predominant species recovered. Our results were in agreement with previous studies (Bautista et al., 1988; Burriel 1998; Pengov, 2001; Gonzalo et al., 2002). In our study CNS isolated were identified by the miniaturized biochemical system (API Staph Biomérieux SA; Marcy-l'Etoile, France). Thought genotypic methods for species identification are to prefer over phenotypic methods in research (Sampimon et al., 2008), on a pratical level they are yet to be routine tests. The role of CNS in increasing SCC has been proved (Pengov, 2001) and they cannot be considered minor pathogens (Gonzalo et al., 2002) as in dairy cows (Devriese and De Keyser, 1980; Boddie et al., 1987). In the present study no significant difference was found in the mean log SCC of half udder infected with CNS when compared with major pathogens (5.79 and 5.64, respectively), while it significantly differed from uninfected glands, where values of 4.57 were reported (Chapter 3), supporting the main role of CNS in increasing SCC in sheep's milk. However, the SCC of udders infected with CNS was lower when compared with values reported in literature (Pengov, 2001). Although SCC do not affect the total casein content of cheese (Revilla et al., 2009), there are evidences that milk with high somatic cell content can negatively affect the cheesemaking process by slowing-down of coagulation (Pirisi et al., 2000) and determination of off-flavour (Jaeggi et al., 2003). The correlation existing between subclinical infection and the high content in SCC of sheep's milk, and on cheese yield, stress the importance of implementing strategies of control of infection in order to improve the hygienic quality of milk. This would reduce the economic losses due to mastitis, increases the economic returns of dairy producers and improve the safety of dairy products. Another

important aspect of a SCC control program is the treatment of mastitis. Dry therapy is a practice that has been proven to be effective in controlling mastitis in dairy cows (Neave et al., 1966; Natzke, 1981; Berry and Hillerton, 2002), and information is available on the administration of intramammary long-acting antibiotic therapy at dry off in dairy ewes, showing a reduction in the prevalence of intramammary infection in the post lambing period (De Santis et al., 2001; Chaffer et al., 2003; Gonzalo et al., 2004). The aim of antibiotic dry therapy is to cure existing infections and to prevent the onset of new ones at parturition (Postle and Natzke, 1974; Eberhart, 1986). The advantage of treating during the dry period is that the antibiotic is used when the sheep are not being milked, and considering the long duration of the dry period, there is no need to discard the milk or possibility of antibiotic residues in the bulk-tank milk (Bergonier et al., 2003). Most of research has been carried out in countries where dairy sheep farming has a long tradition, but still there is not a specific protocol to dry treat dairy small ruminants (Shwimmer et al., 2008). When mastitis is in its subclinical form the percentages of cure rate range between 50% and 82.3% (Watson and Buswell, 1984; Ahmad et al., 1992a; Chaffer et al., 2003; Hueston et al., 1989; De Santis et al., 2001). Few drugs are licensed for use in small ruminants (De Santis et al., 2001; Longo and Pravieux, 2001), so that in many cases, the antibiotic used for the treatment of small ruminants is only labelled for dairy cows (Fox et al., 1992; Mercier et al., 1998; Las Heras et al., 2000; Chaffer et al., 2003; Gonzalo et al., 2004), and no withdrawal period is determined when used off-label in the ewes. It is therefore advisable to use antimicrobial detection test for milk when antibiotic are administered to dry ewes. Two different approaches have been proposed to treat dairy ewes, complete dry treatment (CDT) or selective dry off treatment (SDT). In a complete CDT strategy all glands of animals are treated, whether in the SDT only infected udders are treated. Little information is available on selective intramammary dry treatment in dairy ewes (Gonzalo et al., 1998, 2004; De Santis et al, 2005). Some aspects of sheep

husbandry system, such as the larger herd size, the lower income of sheep producers, the higher average treatment cost per animal, the high rate of spontaneous cure, support the selective rather than complete dry off therapy. As a consequence of a generalized use of antibiotic, some concern exists on the risk of antibiotic residues in milk after dry treatment. Antimicrobial residues may cause allergies to the consumers, or potentially develop antibiotic resistance. The risk of antibiotic residues in sheep milk seems to be almost null, Lohuis et al. (1995), reported no residues in milk after three days of lambing in ewes treated at dry off with a bovine formulation. During the present study bulk tank milk was regularly checked for the presence of antibiotic residues being always below the detection limit. However, antibiotic detection methods for sheep need yet to be standardized (Yamaki et al., 2004; Montero et al., 2005). For all these reasons, a selective dry off treatment approach is advisable for dairy ewes. The glands of the animals requiring antibiotic treatment are selected by a clinical examination or by iSCC (Natzke, 1981; Rindsing et al. 1978). In the USA it is a relatively new sector and little information is available on the effectiveness of antibiotic dry treatment. The study presented in Chapter 3 was aimed to determine the effect of intramammary antibiotic dry treatment given to milking ewes on prevalence of intramammary infection. The gold standard to identify infected glands is bacteriological examination of milk. For economic reasons, in dairy ewes at field-level this type of analysis is not performed on a regular basis, especially in the case of subclinical mastitis. In the present study iSCC, usually available at farm level, was used to select ewes to treat. The use of SCC as a decision rule would give farmers an important tool in managing subclinical infection and improve milk quality. Considering that in dairy ewes there is no agreement on what threshold should be used to differentiate between healthy and infected glands, in fact many cut-off point have been proposed by different authors ranging from 200,000 to 2,000,000 cells/ml (Green, 1984; Mackie and Rodgers, 1986; Maisi et al., 1987; Fthenakis et al., 1991; González-Rodríguez et al., 1995; Mavrogenis et al., 1995; Fthenakis,

1996; Pengov, 2001; McDougall et al., 2001; Berthelot et al., 2006). The variability of SCC depending on the breed, stage of lactation, milk fraction, flock management and so forth, suggest that the use of a dynamical and multiple threshold approach should be used (Bergonier et al., 1996), allowing to classify ewes as infected, healthy and doubtful. Of course a single threshold approach (or punctual approach), with all its limitation, is the simplest method. The study in presented in Chapter 3 was aimed to assess the feasibility of using monthly test day SCC to select ewes to be treated using a threshold of 400,000 cells/ml. The present threshold takes into account the differences existing between ewes and cows and is supported by a previous study (Spanu et al., 2008) where ewes with SCC ≥ 400,000 cells/ml in the last test before dry treatment were more likely to have IMI. The effectiveness of the treatment was evaluated on the prevalence of IMI in the post partum period. Unlike other studies, the present study was not able to show any significant effect of dry treatment on the prevalence of IMI. A possible explanation is that the low prevalence of IMI in the flock might make it difficult to detect the effect of the treatment. Moreover, the present study was characterized by a high prevalence of yeast infections, ranging from 12% to 28%. Fungi (moulds and yeast) are common environmental organisms (Kirk and Bartlett, 1986) and they can be found in different substrates such as soil (Richard et al. 1980), plants, bedding material (Loftsgard and Lindquist, 1960), feed and water (Elad et al., 1995; Hintikka, 1995). They're normally present on the skin of the udder and teats in low numbers (Loftsgard and Lindquist, 1960; Farnsworth and Sorensen, 1972) and act as opportunist pathogens of the mammary gland and cause mastitis when udder natural defence is lowered. Associated factors as trauma from the milking machine, use of irritating teat dips may contribute to develop a yeast infection (Giesecke et al., 1968). Even thought mycotic mastitis is considered to be sporadic condition in favourable circumstances it may occur in epidemic proportion (Schalm et al., 1971; Gonzalez, 1996). The incidence of mycotic mastitis is usually associated with

intramammary infusion of antibiotics when contaminated syringes are used (Paine, 1952; Loftsgard and Lindquist, 1960; Mantovani et al., 1970; Farnsworth and Sorensen, 1972; Thompson et al., 1978; Richard et al., 1980; Kirk and Bartlett, 1986; Krukowski et al., 2000;). Yeast enters the teat canal either by means of inappropriate use of instruments, such as cannulas or syringes, or contaminated antibiotic preparations used for infusion (Sheena and Siegler, 1995). Teat injuries may facilitate a yeast infection (Gonzalez, 1996). Invasion of fungi may also be facilitated by large doses of antibiotic that cause a reduction in the vitamin A content (Kauker, 1955). Yeast infection could be suspected in mild cases that don't respond to antibiotic treatment. Clinical signs usually appears within 10 to 12 days from treatment (Richard et al. 1980). In these cases usually clinical signs disappear completely without therapy in two to four weeks (Richard et al. 1980; Farnsworth, 1977; Loftsgard and Lindquist, 1960). It is difficult to interpret whether the isolation of yeast from a single milk sample is due by contamination or an intramammary infection. A yeast should be isolated several times in succession for a positive diagnosis of yeast infection to be possible (Richard et al. 1980; Hintikka, 1995). Few cases of mycotic mastitis by *Aspergillus fumigatus* have been reported in small ruminants (Berthelot and Bergonier, 1993; Jensen et al., 1996; Pérez et.al, 1998). An association between mammary aspergillosis and incorrect administration of antibiotic at drying-off was suggested (Las Heras et al., 2000). All the yeast infection reported were found in the dry treated group, suggesting a lack of hygienic conditions during administration of intramammary DT. This may have affected the efficacy of the antibiotic dry treatment observed in this study. This finding further confirms the importance of performing antibiotic dry therapy under proper hygienic conditions, using sterile products and equipment and following proper sanitary procedures. Another fact that might have influenced the effect of the treatment is the high overall self-cure rate that ranges between 35.0 to 67.0% in small ruminants (Watson and Buswell, 1984; Hueston et al., 1986, 1989; Ahmad et al., 1992b; Paape

et al., 2001; Bergonier and Bethelot, 2003). A decline of IMI with time post partum regardless the dry treatment was observed, this in agreement with other author's findings (Hueston et al., 1986; Kirk et al., 1996; McDougall et al., 2002), but other studies reported different observations, with an increase of cases (Al-Samarrae et al., 1985; Fthenakis, 1994; Watson et al., 1990; Watkins et al., 1991). Increasing prevalence of IMI during the time post partum is expected due to an increased exposure to pathogens as the lactation proceeds. Although one might consider that differences exist in the different surveys concerning the husbandry location, the environment, the breed, the definition of a case, sampling strategies, experimental design, diagnostic techniques, and management practices. In the same fashion, older ewes have an increased risk of developing mastitis (Fthenakis, 1988, 1994; Watson et al., 1990; Watkins et al., 1991). Whereas in this study a different trend was observed, prevalence of IMI was not associated with parity. Similar finding was reported by McDougall et al. (2002).

When the effect of DT was evaluated on mean SCC in the subsequent lactation (Chapter5) its effectiveness was evident. The $\log_{10}$ SCC was greater in the not treated group (5.0) as compared to the dry treated (4.8) indicating the effectiveness of the antibiotic dry treatment in lowering the SCC. These results are in agreement with other authors (Linage and Gonzalo, 2008) who evaluated the efficiency of dry ewe therapy in improving milk SCC at lambing. The importance of SCC history as predictor was demonstrated either on the prevalence of IMI (Chapter 3) and on the mean log10 SCC in the subsequent lactation (Chapter 5). In fact, the ewes with ≥ 3 monthly test with SCC ≥ 400,000 cells/ml were up to 7.5 times more likely to have mastitis infections in the period postpartum when compared to ewes with <3 test days with high SCC (Chapter3). A significant difference was reported (Chapter 5) in the mean $\log_{10}$SCC of ewes with a history of high SCC (more than 3 test in the previous lactation greater

or equal to 400,000 cells/ml). This further confirms the value of using SCC reports as a mastitis management tool.

A well established practice adopted in dairy cows to reduce bacterial contamination on the teat skin and as a consequence the risk of intramammary infection is teat disinfection (Philpot and Pankey, 1975; Natzke, 1977; Philpot, 1979; Farnsworth, 1980; Pankey et al., 1984; Galton et al. 1986; Rasmussen et al. 1991; Oliver et al. 1993; Bramley et al., 1996; Nickerson, 2001; Magnusson et al. 2006). Little information is available on the effect of teat sanitation in dairy ewes. In the present study (Chapter 4) was to assess the impact of iodine premilking teat sanitation on somatic cell count in dairy sheep. Predipping is intended to combat environmental pathogens by reducing microbial population on teat skin before milking. The use of predipping in dairy ewes should be justified by the consideration that the prevalence by contagious pathogens (*S. aureus* and *Streptococcus agalactiae*) is low. Although CNS are normally present on teat surface and they elicit high SCC levels, no significant effect of sanitation on SCC levels was found in this study. Previous research in dairy cattle has demonstrated that predipping is not always cost effective when CNS are the predominant mastitis pathogens (Ruegg and Dohoo, 1997). Teat sanitation was effective in ewes with increased risk (history of high somatic cell count and older parity group), indicating an eventual application, but further research is needed to evaluate the effect of premilking teat sanitation on reduction of bacterial count and on the incidence of intramammary infections.

Conclusion

Hygienic production of milk is paramount for all the dairy sheep industry (farmers, cheese processors, consumers and official control). Bacterial count and somatic cell count are the criteria taken into account in evaluating milk quality. Hygienic production of milk goes further than simple hygienic milking. Farmers should put into place a complete milk quality control program including well designed procedure to apply on a regular basis on their farm. Within these actions to control udder health are: a correct diagnosis of animals with clinical and subclinical mastitis, drying off therapy, hygienic milking routine practices (teat dipping) and culling. Improving udder health is the key point of obtaining hygienic milk and to assure a high level of protection of human health. Many factors should be taken into account before a comprehensive dry off treatment is applied. Due to the large size of the flocks, the low income of sheep farming, the relatively low price of the animal compared with the culling cost, make it necessary an accurate evaluation of the cost and benefit of such treatment. The cost to treat dairy ewes is high when compared with the culling cost of the animal, so as a general rule complete dry off treatment strategies are to prefer with high prevalence flocks, whereas selective dry off therapy should be preferred with low prevalence. The use of iSCC history as a decision rule is an effective tool in farmer's hands to select animal to treat, even without bacteriological culture. Bulk tank and individual milk can be checked for SCC with animal side tests, such as CMT or DCC, helping in controlling and managing milk quality on one side and public health on the other. Though dry treatment is an important element to improve milk quality, it could be a potential source of antibiotic residues in milk. Considering the long dry off period in ewes, this risk is considered almost null. Another potential concern of antibiotic treatment is the development of antibiotic resistance by pathogens. A prerequisite essential for the treatment to be effective is to respect hygiene condition during antibiotic administration. Our results suggest that when ewes have recurrent high somatic cell counts during the lactation they're more likely to have high somatic cell count in the subsequent

lactation regardless the dry treatment. In such cases might be advisable considering culling instead of treatment. Teat dip before milking with a sanitizing solution is a good practice to reduce the bacterial load on the teat skin, but before it is suggested as a routine its efficacy in preventing new IMI in small ruminants further research is needed.

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