Genetic aspects of somatic cell count and udder health in the Italian Valle del Belice dairy sheep

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Abstract

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This thesis is part of a project supported by the Ministero delle Politiche Agricole e Forestali (MiPAF) for improving udder sanitation and mastitis control in the Valle del Belice dairy sheep breed. Mastitis is an inflammation of the udder, which leads to economic loss, mainly consisting of discarded milk, reduced milk production and quality, and increased health costs. Somatic cell count (SCC), and therefore somatic cell score (SCS), is widely used as indicator of mastitis. In this thesis, I focus on the genetic parameters of SCS as indicator of mastitis, and on the possibilities of using this trait for selection for mastitis resistance in the Valle del Belice dairy sheep.
In Chapter 1, mastitis and SCS are defined and introduced. Chapter 2 deals with the estimation of genetic parameters for SCS and milk production traits in primiparous Valle del Belice ewes. Heritability estimates ranged from 0.09 to 0.14 for milk, fat and protein yields and contents. For SCS, the heritability of 0.14 was relatively high. SCS was genetically positively correlated to milk, fat and protein yields and contents. However, correlations were not extreme, so simultaneous improvement for milk yield and SCS seems possible. In Chapter 3, the level of SCC is included in a survival analysis to evaluate the effect of SCC on functional longevity. Results showed that an increase in SCC was associated with an increase in culling rate. Elevated SCC, therefore, play an indirect role in the culling decisions of Valle del Belice dairy sheep farmers, although, at present, farmers do not directly select for reduced SCC. In Chapter 4, the genetic parameters of the infection status and SCS, according to whether the samples were bacteria negative or positive are reported. Moreover, the impact of imperfect sensitivity and specificity on variance component estimates was investigated. The heritability was 0.10 for bacteria negative SCS, 0.03 for bacteria positive SCS, and 0.09 for infection status, on the liability scale. The genetic correlation between bacteria negative and bacteria positive SCS (0.62) suggests that they may be genetically different traits, confirming that SCS from healthy and infected animals should be analyzed separately. Moreover, a positive genetic correlation between bacteria negative SCS and liability to mastitis was found, suggesting that the approach of selecting animals for decreased SCS will help to reduce the prevalence of mastitis. The results also showed that the imperfect diagnosis of infection has an impact on estimated genetic parameters, which may reduce the efficiency of selection strategies aiming at distinguishing between bacteria negative and bacteria positive SCS. In Chapter 5,

the diagnostic ability of SCC and California Mastitis Test (CMT) to detect intramammary infections was evaluated by using the Receiver-Operating Characteristic curves, in order to identify a SCC threshold that better discriminated healthy from infected udders. Three different SCS traits were considered: SCS for the whole sample (i.e., considering uninfected and infected glands), SCS for minor pathogens (i.e., considering uninfected and infected by minor pathogens glands), and SCS for major pathogens (i.e., considering uninfected and infected by major pathogens glands). The results indicate that the CMT can only discriminate the udders infected from major pathogens. Nevertheless, in general SCS was the best indirect test for the bacteriological status of the udder.

The final chapter explores and discusses the opportunities to use SCS as indicator of mastitis in a selection scheme to improve mastitis resistance for the Valle del Belice dairy sheep breed.

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General introduction

1.1 Introduction

The Mediterranean Basin countries host 60% of the total world sheep and goat milk production. The dairy sheep and goat industry is usually based on local breeds, which are very well adapted to their production systems and environments. When compared to other species, dairy sheep and goats have some specific features that affect the structure and potential genetic progress of a breeding program. Dairy sheep and goats usually show marked reproductive anoestrous (i.e., seasonal production pattern); they are dual purpose, where part of the income comes from lamb and kid meat, and milking usually starts after an average suckling period of 30 days. Moreover, the use of artificial insemination is limited and natural mating still plays an important role in reproduction, which compromises correct parentage assignment.

Milk production is the principal trait affecting profitability of dairy sheep and goat industry, and therefore breeding programs mainly focus on milk production traits. However, due to the EU agricultural policy and consumer demand, recently increased attention is for traits related to the reduction of production costs, food safety and health (e.g., resistance to intramammary infections, IMI). In particular, mastitis is one of the primary intramammary infections in dairy sheep and goats as well as in dairy cattle. Bergonier and Berthelot (2003) reported that annual incidence of clinical mastitis in sheep is generally lower than 5%, whereas the incidence of subclinical mastitis ranges from less than 10 to 50% or more.

Mastitis is an inflammation of the udder, generally caused by bacteria, and it leads to economic loss, mainly consisting of discarded milk, reduced milk production and quality, and increased health costs (i.e., Miller et al., 1993; Allore and Erb, 1998; Leitner et al., 2003). Moreover, Legarra et al. (2007) reported that susceptibility to mastitis is one of the reasons for culling in sheep. Barillet et al. (2001) reported a 5% frequency of culling due to clinical mastitis and a 10% frequency due to subclinical mastitis.

Mastitis resistance is a complex trait, depending on both genetic and environmental factors, including infection pressure. In the broadest sense, resistance could be defined as the ability to avoid any infection and/or the quick recovery from an infection (Rupp and Boichard, 2003), and it involves different components: avoiding entry of the pathogen into the mammary gland, mounting an immune response capable of limiting its development in the udder and clearing the infection, as well as controlling the pathogenic effects of the infection, such as tissue damage (Rupp et al., 2010). Selecting for increased genetic resistance to mastitis can be done directly or indirectly. Direct selection corresponds to the

diagnosis of the infection: the actual trait (i.e., bacteriological examination of milk and/or observation of clinical cases of mastitis) is measured on the animal or its relatives. Indirect selection corresponds to a prediction of the bacteriological status of the udder based on traits related to the infection (e.g., inflammatory parameters): in this case, an indicator trait for mastitis is measured on the animal itself or its relatives (de Haas, 2003).

Direct bacteriological assay is the recommended method of diagnosis of mastitis (González-Rodríguez and Cármenes, 1996), because it is believed to provide precise and exhaustive information on infected quarters and pathogen involved. However, it is rarely used for genetic purpose, because it is difficult to implement at a large scale and it has limitations because of the requirement of intensive labour, the time delays for culture to occur, and the costs associated with bacteriology (McDougall et al., 2001). Moreover, it has been shown that bacterial shedding is variable and levels may sometimes be too low to be detected by conventional techniques (Rupp et al., 2010). Simple, indirect methods have been widely applied, based on the evaluation of the degree of inflammation or of internal mammary lesions (De la Cruz et al., 1994). Their accuracy is established by bacteriological analysis as a reference method. Among the methods, the most frequently used to detect mastitis are milk somatic cell count (SCC), the California Mastitis Test (CMT), and electrical impedance.

This chapter provides a review on mastitis and mastitis-causing pathogens, highlighting the differences among the main livestock dairy species (i.e., cattle, sheep and goats) and what is already known on the use of SCC for selection for mastitis resistance; it also provides a description of the Valle del Belice breed and its production environment, underlying the importance of this breed for the Sicilian farmers' economy; it finally indicates the aim and outline of the thesis.

1.2 Mastitis and mastitis-causing pathogens

Mastitis can be classified as subclinical or clinical. Mastitis is subclinical when no visible changes occur in the appearance of both milk and udder, but milk production decreases, bacteria are present in milk and the milk composition is altered (Harmon, 1994). On the other hand, mastitis is clinical when symptoms such as fever, abnormal texture and discoloration of the milk, increased temperature or pain of the quarter, and change in milk properties occur.

Generally, the incidence of clinical mastitis varies between 20 and 40% per cow/year (Heringstad et al., 2000); whereas the annual incidence of clinical mastitis in small ruminants is generally lower than 5% (Contreras et al., 2007). The

incidence of subclinical mastitis in sheep and goat has been estimated at 5-30% per lactation or even higher (Bergonier and Berthelot, 2003; Contreras et al., 2003). Mastitis in dairy sheep results mainly from bacterial infections whose reservoir is generally in the udder or teat and transmission between ewes is increased by milking (Lagriffoul et al., 2006).

Over 100 different micro-organisms can cause mastitis, in particular coliform bacteria, staphylococci and streptococci (Smith and Hogan, 2001), in both cattle and small ruminants. The bacterial pathogens responsible for infection of the mammary gland may be split into two main categories: major and minor pathogens. Infection with major pathogens generally results in clinical illness or strong inflammatory responses and reduced milk yields, whereas minor pathogen infection is usually subclinical (White et al., 2001). Pathogens can be also categorised depending on their aetiology into environmental and contagious (e.g., Fox and Gay, 1993):

- environmental bacteria (found in the soil, faeces, and bedding), which enter the teat duct from these sources; they include both Gram-positive and Gram-negative bacteria such as *Streptococcus* non-agalactiae and coliform organisms (*Escherichia* coli, *Klebsiella* sp., *Aerobacter* aerogenes, *Enterobacter* sp.);
- contagious bacteria, which are transmitted from infected quarters to uninfected quarters during the milking process and include such Gram-positive bacteria as *Staphylococcus* aureus and *Streptococcus* agalactiae.

Coagulase-negative staphylococci (CNS) are the most common bacterial species that cause mastitis in sheep breeds (e.g., Albizu et al., 1991; Marco et al., 1991) and produce not only subclinical but also clinical mastitis (Amorena et al., 1991). Gonzalo et al. (1998), therefore, suggested dividing the CNS into two groups with different pathogenicity among dairy sheep: NRCNS (novobiocin-resistant CNS), which behave as minor pathogens, resulting in mild changes in somatic cells and milk yield, similar to those commonly associated with micrococci and corynebacteria (i.e., Ziluaga et al., 1998), and NSCNS (novobiocin-sensitive CNS), which cause more substantial changes in SCC and milk yield loss, similar to those associated with classic major pathogens (Peris et al., 1996).

1.3 SCC and mastitis

Somatic cells occur normally in milk of both cattle and small ruminants. Somatic cells consist of many types of cells, including polymorphonuclear leukocytes (PMN), macrophages, lymphocytes, eosinophils, and various epithelial cells from the mammary gland. Cells in milk from a healthy udder are mainly represented by

mammary gland epithelium and drain canal cells. In cattle, only 8% are leukocytes and less than 1% are macrophages (Walawski, 1999). PMN are the major cell population during early inflammation; they play a protective role against infectious diseases in the mammary gland (i.e., Kherli and Shuster, 1994; Persson-Waller et al., 1997). In mammary glands of cattle infected with mastitis-causing pathogens, milk somatic cells consist for more than 95% of PMN, and PMN are thus indicators of inflammatory response (Detilleux et al., 1997). Experimental intramammary infection of sheep with *Staphylococcus* aureus or *Escherichia* coli has been shown to induce a significant increase in PMN within 24 h of infection (Persson-Waller et al., 1997).

The concentration of somatic cells in milk is defined as somatic cell count (SCC) and it is expressed as thousands of cells per millilitre of milk. The measure of SCC has the following properties:

- it can be routinely recorded in most milk recording systems;
- the heritability of SCC is higher than the heritability of the direct trait (i.e., mastitis incidence);
- it is an indicator for both clinical and subclinical infections.

The distribution of SCC is positively skewed; whereas, conventional statistical methods usually assume normally distributed data. In order to obtain a distribution which closely resembles a normal distribution, the SCC is log-transformed to somatic cell score (SCS). The formula widely used is:

$SCS = \log_2(SCC/100) + 3$ (Ali and Shook, 1980).

In genetic programs, SCS is assumed to be genetically correlated linearly with mastitis and other traits. This approach implicitly assumes that SCC of both uninfected and infected animals follow the same distribution. However, it has been reported that SCC from uninfected and infected animals should be considered as different traits (Detilleux and Leroy, 2000; Heringstad et al., 2003; Ødegård et al., 2003). A difference between the two traits (i.e., SCC from uninfected and infected animals) has been recently confirmed in the distribution of SCC values in cattle (ten Napel et al., 2009). These authors also provided evidence that minor and major pathogens result in different distributions of SCC. Moreover, SCC diagnostic capability (i.e., SCC ability to detect whether or not an infection occurs) may be assessed without having to commit to a single threshold with receiver-operating characteristic (ROC) curves. An ROC curve is a plot of a test's true-positive fractions (TPF) versus false-positive fractions (FPF) for each possible test result (Hanley and McNeil, 1982), indicating all tradeoffs between sensitivity and specificity that are available.

1.3.1 SCC in cattle

SCC is considered as a good measure to select indirectly for mastitis resistance in cattle, especially when a direct measure of clinical mastitis incidence is not available (Shook and Schutz, 1994; Colleau and Le Bihan-Duval, 1995; Heringstad et al., 2000). The direct selection to reduce the incidence of mastitis is indeed the current practice only in the Nordic countries (Denmark, Finland, Norway, and Sweden).

It has been suggested that milk obtained from a normal healthy cow contains less than 10^5 somatic cells per mL (i.e., Kherli and Shuster, 1994). Other authors, however, recommended values between 250 and 300 x 10^3 cells/mL as a most satisfactory discrimination threshold between healthy and infected udders in cattle.

The use of SCC for selection purposes has been widely discussed and it has been shown that selection for decreased SCC would reduce susceptibility to mastitis (Philipsson et al., 1995). Moreover, Philipsson et al. (1995) estimated a linear relationship between SCC and the occurrence of clinical mastitis, concluding that selection for lower SCC is desirable and that a lower level of SCC reflects a reduced incidence of infection, rather than a reduced ability to react to it. Therefore, SCC should be decreased to the lowest possible value. This agrees with the results of Rupp et al. (2000), who concluded that cows with the lowest mean SCC in the first lactation had the lowest risk for clinical mastitis in the second lactation. These results, therefore, suggest that genetic selection for decreased SCC may effectively reduce incidence of clinical mastitis and that breeding goals should favour animals with the lowest observed SCC. However, it might be necessary to monitor if this does not affect the ability to resist infections.

A large number of estimates of heritabilities and variance components for SCC and/or SCS are reported in the literature for cattle, using either test-day or lactation models. Heritability estimates for single monthly test-day SCS range from 0.05 to 0.14 (i.e., Carnier et al., 1997; Mrode et al., 1998). Heritability estimates tend to increase slightly from the beginning to the end of the lactation due to a constant genetic variance and a decreasing environmental variance (Rupp and Boichard, 2003). The lactation measure of SCS, obtained by averaging the individual test-day records, shows a consistent higher heritability estimate with a range from 0.10 to 0.18 (i.e., Rupp and Boichard, 1999).

Mastitis incidence is considered to have an important effect on culling decisions in dairy cows (Beaudeau et al., 1994; Gröhn et al., 1998; Neerhof et al., 2000), particularly mastitis occurring before the peak of lactation (Beaudeau et al., 1994). Correlations between breeding values for longevity and mastitis resistance, ranging from 0.22 to 0.33, were found in dairy cows (Nielsen and Pedersen, 1995). The

effect of SCC on culling at the phenotypic level was first assessed by Beaudeau et al. (1995). Higher SCC were associated with higher rates of culling. Moreover, a negative genetic correlation of -0.32 between SCC and herd life was estimated by Mrode et al. (2000), indicating that a high SCC was associated with reduced longevity in cattle.

Genetic correlations between SCC and incidence of clinical mastitis in cattle vary from moderate to high with an average around 0.7 (for a review, Mrode and Swanson, 1996). These results, therefore, confirm that, although SCC and mastitis are not the same trait, SCC can be used as a selection criterion in a breeding programme for mastitis resistance in cattle.

1.3.2 SCC in goat

Goat milk contains on average higher SCC than cow milk, as the physiology of the caprine mammary gland is distinct from the bovine one, secreting non-cellular particles that can be mistakenly counted as somatic cells (Contreras et al., 1996): goat as well as human mammary epithelium secrete proteins by an apocrine mechanism gland cells (Schalm et al., 1971), a process by which epithelial cell cytoplasmic projections are released into the lumen of the *acini* resulting in cytoplasmic particles which become part of the normal composition of the milk, compared to the cow for which the mammary gland is a merocrine organ with relatively few cytoplasmic particles in the normal milk (Pantschenko et al., 2000). This has long been a concern of goat owners because of regulatory standards and marketing problems. SCC has been reported to range from 360 to 880 x 10^3 cells/mL in milk of uninfected goats (Poutrel and Lerondelle, 1983; Manser, 1986). Wilson et al. (1995) showed that more than 90% of the variation in SCC in goats was not due to intramammary infections. However, this result is not in agreement with that of Corrales et al. (2004), who reported that clinical intramammary infections by *Mycoplasma* was one of the most important causes of elevated SCC in goat milk.

Milk yield losses and increased SCC in infected udders have been documented in both goats (e.g., Leitner et al., 2004a) and sheep (e.g., Gonzalo et al., 1994; Leitner et al., 2004b), and it appears that sheep are more vulnerable than goats to milk yield losses due to subclinical mastitis (Silanikove et al., 2005). However, to my knowledge no estimates of genetic correlations between SCC and clinical and subclinical mastitis incidence have been reported for dairy goats.

1.3.3 SCC in sheep

Whereas in cattle values of SCC between 250 and 300 x 10^3 cells/mL are recommended as most satisfactory discrimination thresholds between healthy and infected udders, in sheep there is not a widely accepted threshold. Some evidence has been provided that healthy ewes have normally higher SCC than cows (i.e., Maisi et al., 1987; Fthenakis et al., 1991; González-Rodríguez et al., 1995). Bufano et al. (1996) showed that high SSC (>1 million/mL) do occur in healthy sheep and goat milk, especially towards the end of lactation.

On the other hand, considering subclinical mastitis, Leitner et al. (2008) suggested that, whereas in dairy cows subclinical mastitis is largely ignored because the increase in SCC in infected glands is modest (about 300-500×10^3 cells/mL) and the mixing with the milk from uninfected quarters is sufficient in most cases to appreciably lower the effect of SCC at the cow level, in sheep and goats, which have only two mammary glands, mixing of milk with high SCC coming from an infected gland with low SCC from a healthy gland might be insufficient to reduce the SCC at the animal level. However, whether these high SCC are a consequence of the fairly generalized lack of preventive management measures against subclinical mastitis in sheep flocks or whether a higher cell discrimination threshold is required for sheep milk has not been established. Some authors (i.e., Fthenakis et al., 1991; Jones, 1991) reported discrimination values between healthy and infected glands ranging from 500 to 1600 x 10^3 cells/mL, but others (i.e., De la Cruz et al. 1994; Pengov, 2001) reported values similar to those for cows (200 to 300 x 10^3 cells/mL).

Genetic studies of SCC in dairy sheep are more recent and less frequent than in dairy cattle. The genetic studies available are mainly limited to the Churra (Baro et al., 1994; El-Saied et al., 1998 and 1999) and Lacaune (i.e., Barillet et al., 1999; Rupp et al., 2001; Rupp and Boichard, 2003) breeds and the estimates were usually based on the average SCS during the lactation. Results based on repeatability test-day models for SCS, indicated heritability estimates ranging from 0.04 for the Churra breed (Baro et al., 1994) to 0.16 for the East Friesian breed (Hamann et al., 2004). Other studies reported higher heritability estimates for the average SCS during lactation, from 0.11 to 0.18 (Mavrogenis et al., 1999; Barillet et al., 2001; Rupp et al., 2001).

Moreover, at present information in dairy sheep regarding the relationship between SCC and longevity is lacking. The current milk payment system of most countries is based only on milk yield and not on SCC level, which is different from the cattle industry. However, it has been reported in sheep that with increasing

SCC, milk pH, whey protein, fat contents, rennet clotting time, and rate of clot firming time rise, whereas lactose, casein contents, and clot firmness decrease (Diaz et al., 1996). Moreover, Giaccone et al. (2005) confirmed that SCC has a considerable influence on the bulk milk composition and lactodynamographic parameters.
No estimates of genetic correlations between SCC and clinical and subclinical mastitis incidence in dairy sheep have been reported in literature.

1.4 Origin and description of the Valle del Belice dairy breed

The *Valle del Belice* dairy sheep (Figure 1.1) originates from western Sicily and its name is derived from the Belice valley, delimited by the Sicilian provinces of Palermo, Agrigento and Trapani.

Figure 1.1 Valle del Belice sheep.

This breed is considered to originate from a three way cross between the *Pinzirita*, *Comisana* and *Sarda* dairy breeds (Portolano, 1987). The *Pinzirita* breed, a native Sicilian sheep found in the western part of Sicily (Portolano et al., 1996), was first crossed with the *Comisana* dairy breed, which originated in the south-east of Sicily (Portolano, 1987). Crosses between the *Pinzirita* and *Comisana* breeds gave birth to

individuals having intermediate characteristics between parental lines. These animals were crossed with sheep belonging to the *Sarda* dairy breed, imported to Sicily during the Arab domination (~800 a.D.) of the island (Portolano, 1987).
In the nineties, data collected by the Sicilian Farmer Association (ARAS) for milk recording, and morphological measurements collected by the University of Palermo, allowed the development of the *Valle del Belice* breed standard. This was submitted in 1996 to both the Dairy Sub-Committee and the Ewes Technical Committee. In 1997 the *Valle del Belice* breed was given official recognition.
The Valle del Belice breed is mainly used for milk production. Average milk production is 139 $\pm$ 35 liters in the first lactation, and 210 $\pm$ 62 liters for later lactations (AIA, 2006). Fat and protein contents are 6% and 5.5% respectively. The head is fine and extended and the trunk well developed with good transversal diameters. A white coat covers the entire body with the exception of limbs, belly and head. Typical are the reddish brown spots surrounding the eyes and on the last part of the ears.

Figure 1.2 Typical farm of Valle del Belice sheep.

Management of the Valle del Belice breed is characterized by the enormous variability. A typical family farming system is conducted and the breed is mainly raised under semi-extensive grazing conditions (Figure 1.2). Ewes are milked twice

a day (morning and evening), and are housed in old storehouses or kept in fenced-in enclosures after the evening milking.
Most of the farmers milk ewes by hand but some of the farms use a milking machine. Furthermore, the lambing system is different from the one adopted in other Mediterranean regions (e.g., Carta et al., 1995; Ligda et al., 2000). The lambing season of the Valle del Belice breed is all year long, starting in July and finishing in the following June, with few lambings in May and June. The primiparous ewes usually give birth between December and March. Moreover, sheep are fed natural pastures and fodder crops; supplementation, consisting of hay and sometimes concentrates, is occasionally supplied, for example at the end of gestation (Cappio-Borlino et al., 1997). The grazing possibilities, the chemical and nutritional composition of the feed, change annually and also differ between areas.
The main use of the milk from Valle del Belice sheep is for the production of traditional raw milk cheeses (pecorino and vastedda del Belice cheeses), at farm level or by small local dairies or by cheese industries working at regional level. Mastitis represents, therefore, one of main issues for Valle del Belice farmers, especially considering that a prevalence of up to 35% has been reported. This has led the Ministero delle Politiche Agricole e Forestali (MiPAF) to financially support research towards improved udder sanitation and mastitis control in this breed.

1.5 Aim and outline of this thesis

The aim of this thesis is to analyse genetic aspects of SCC in Valle del Belice dairy sheep, in order to study the use of SCC data in genetic selection for mastitis resistance. Chapter 2 deals with the estimation of heritabilities for SCS and milk production traits in primiparous Valle del Belice ewes, using a repeatability test-day animal model. Moreover, in the same chapter, the phenotypic and genetic correlations of SCS with milk production traits are reported. In Chapter 3, the level of SCC is included in a survival analysis to evaluate the effect of SCC on functional longevity. In Chapter 4, the heritabilities of SCS, according to whether the samples were bacteria negative or positive are reported as well as the genetic correlations between bacteria negative and bacteria positive SCS, and between bacteria negative SCS and the infection status. The main aim of the research in this chapter was to get an idea whether we need to worry about reducing baseline SCC levels. Moreover, the impact of imperfect sensitivity and specificity on variance component estimates for the traits of interest was investigated. In Chapter 5, the diagnostic ability of SCC was evaluated by using the ROC curves, in order to identify a SCC threshold that better discriminated healthy from infected udders. A General

Discussion follows (Chapter 6), which explores the opportunities to use SCC as indicator of mastitis in a selection scheme for mastitis resistance for the Valle del Belice dairy sheep breed.

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2

Genetic parameters for milk somatic cell score and relationships with production traits in primiparous dairy sheep

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Abstract

A total of 13,066 first lactation test-day records of 2,277 Valle del Belice ewes from 17 flocks were used to estimate genetic parameters for somatic cell scores (SCS) and milk production traits, using a repeatability test-day animal model. Heritability estimates were low and ranged from 0.09 to 0.14 for milk, fat and protein yields and contents. For SCS, the heritability of 0.14 was relatively high. The repeatabilities were moderate and ranged from 0.29 to 0.47 for milk production traits. The repeatability for SCS was 0.36. Flock-test-day explained a large proportion of the variation for milk production traits, but it did not have a big effect on SCS. The genetic correlations of fat and protein yields with fat and protein percentages were positive and high, indicating a strong association between these traits. The genetic correlations of milk production traits with SCS were positive and ranged from 0.16 to 0.31. The results showed that SCS is a heritable trait in Valle del Belice sheep and that single-trait selection for increased milk production will also increase SCS.

Key words: somatic cell count, milk production, genetic parameter, dairy sheep

2.1 Introduction

Mastitis is one of the major diseases in dairy cows and ewes and has motivated extensive research toward improved udder sanitation and mastitis control (El-Saied et al., 1998). Selection for improved resistance to mastitis can be done directly, by selecting against mastitis itself or indirectly by selecting for a trait correlated with mastitis (e.g., De Haas, 2003). In particular, somatic cell count (SCC) has been promoted as an accurate indirect method to predict subclinical or clinical mammary infections in dairy cattle and in dairy sheep. As such SCC has also been suggested as selection criterion for mastitis resistance (Colleau and Le Bihan-Duval, 1995). It has been demonstrated that the occurrence of mastitis causes an increase in somatic cells (e.g., Sordillo et al., 1996). Hence, milk with an elevated SCC is an indication of the occurrence of infection in the udder; and selection for decreased SCC could lead to a reduction in susceptibility to mastitis (e.g., Mrode and Swanson, 1996).

Genetic parameters for SCC are required to study possibilities of changing SCC by means of selection. Commonly, SCC is log-transformed to SCS. Genetic studies of SCS in dairy sheep are more recent and less frequent than in dairy cattle. The few available genetic studies are on a limited number of breeds, e.g., on the Churra (Baro et al., 1994; El-Saied et al., 1998) and Lacaune (Barillet et al., 2001; Rupp et al., 2001 and 2003) breeds and the estimates are usually based on average SCS during the lactation. Furthermore, information on the genetic relationship between SCS and milk yield and composition is lacking, and no references are reported in literature on dairy sheep reared in the south of Mediterranean area, where the husbandry system and the management are very different from those adopted for the breeds reared (i.e., Lacaune and Churra) in the north of the Mediterranean area.

The aim of this study was to evaluate genetic aspects of SCS and the relationships between SCS and milk yield, and fat and protein contents and yields in primiparous Valle del Belice ewes using a repeatability test-day animal model (Ptak and Schaeffer, 1993).

2.2 Material and methods

The original data set used for this study included 16,883 records of 3,004 primiparous ewes. Data were collected by the University of Palermo between 1998 and 2003 in 17 Valle del Belice flocks. Test-day records of milk yield, fat percentage, fat yield, protein percentage, protein yield, and SCC were collected at approximately monthly intervals, following an A4 recording scheme (ICAR, 1992).

All ewes were milked twice daily, and the milk of both daily milkings was analyzed. Fat and protein percentages and SCC were calculated as the weighted average of the morning and evening milking, where weighting is according to the corresponding milk yield.

Records were removed when ewes had an abortion and when both of the ewe's parents were unknown. After editing, the data set consisted of 13,066 observations on 2,277 ewes. The pedigree file consisted of 4,369 animals; in addition to the 2,277 animals with records, 246 sires and 1,846 dams were included. On average, the sires served at least 2 of the 17 flocks under study and they had 11.34 daughters.

The average number of milk production records per ewe was 5.74 and the average number of SCC test-days per ewe was 5.24. Test-day SCC were converted to SCS using a base 2 logarithmic function: $SCS = \log_2 (SCC/100) + 3$ (Ali and Shook, 1980).

The test-day traits analyzed as response variables were milk yield, fat and protein percentages and yields, and SCS. Variance components and genetic parameters for each trait were estimated using ASREML (Gilmour et al., 2002). Several models were tested to explore the fitted factors and to optimize the analysis; the repeatability test-day animal model reported below was the model with the highest coefficient of determination:

$$y_{ijklm} = \mu + FTD_i + YPS_j + LS_k + \beta_1 DIM_{ijklm} + \beta_2 \exp\left(-0.05 * DIM_{ijklm}\right) + A_l + PE_l + e_{ijklm}$$

where y_{ijklm} is the test-day trait's measurement; μ is the population mean; FTD_i is the random effect of flock by test-day interaction *i* (626 levels); YPS_j is the fixed effect of year by season of lambing interaction *j*, where the season of lambing was equal to 1 if a ewe gave birth in the period January through June, otherwise it was equal to 2 (11 levels); LS_k is the fixed effect of litter size class *k* (2 levels, single or multiple born lambs); DIM_{ijklm} and $\exp\left(-0.05 * DIM_{ijklm}\right)$ are two covariates used to model the shape of lactation curves (Wilmink, 1987), where DIM is the number of days in milk; A_l is the random additive genetic effect of the individual *l* (4,369 levels); PE_l is the random permanent environmental effect on the individual *l* (2,277 levels); e_{ijklm} is the random residual effect.

All known relationships among individuals were considered in the animal model. Heritabilities (h^2) and repeatabilities (r) were calculated as:

$$h^2 = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_{E_p} + \sigma^2_{E_t}} \quad , \quad r = \frac{\sigma^2_A + \sigma^2_{E_p}}{\sigma^2_A + \sigma^2_{E_p} + \sigma^2_{E_t}}$$

where σ_A^2 is the additive genetic variance, $\sigma_{E_p}^2$ is the permanent environmental variance and $\sigma_{E_t}^2$ is the temporary environmental variance.

Bivariate analyses were used to estimate phenotypic and genetic correlations. The model was the same as for the univariate analyses. Estimated variance components from the univariate analyses were used as starting values for the bivariate analyses.

2.3 Results

Means, standard deviations, and coefficients of variation of the test-day traits are given in Table 2.1. The daily average milk yield was 1,167 g, fat yield was 76.1 g and protein yield was 62.6 g. The mean SCC was 1,484 (x 10^3 cells/mL), and the mean SCS was 6.89.

Table 2.1 Descriptive statistics[1] of test-day traits.

Daily Measurements	Mean	SD	CV (%)
Milk Yield (g)	1,167	592	51
Fat (%)	6.80	1.47	22
Fat Yield (g)	76.1	38.1	50
Protein (%)	5.48	0.75	14
Protein Yield (g)	62.6	30.8	49
Somatic Cell Count (x10^3 cells/mL)	1,484	3,648	246
Somatic Cell Score	6.89	2.17	31[2]

[1]Based on 13,066 records for milk production traits and on 11,938 records for somatic cell count and somatic cell score.
[2]The constant is not subtracted from Ali and Shook's formula to calculate the CV for SCS.

The coefficients of variation for milk, fat and protein yields were around 50%. The coefficients of variation for fat and protein percentages were considerably lower. The coefficient of variation for SCC was 246%, whereas the coefficient of variation for SCS was 31%.

Phenotypic variances after adjustment for fixed effects and FTD, the heritabilities, the repeatabilities, and the proportions of variance due to FTD are in Table 2.2.

Table 2.2 Phenotypic variance, heritability, repeatability and flock-test-day (FTD) fraction (± s.e.) for test-day variables.

Trait	σ^2_p [1]	h^2 ± s.e.	r ± s.e.	FTD[2] ± s.e.
Milk Yield (g)	130617	0.12 ± 0.03	0.47 ± 0.01	0.52 ± 0.02
Fat (%)	0.95	0.09 ± 0.02	0.29 ± 0.01	0.46 ± 0.02
Fat Yield (g)	594	0.14 ± 0.03	0.44 ± 0.01	0.51 ± 0.02
Protein (%)	0.29	0.14 ± 0.03	0.41 ± 0.01	0.36 ± 0.02
Protein Yield (g)	345	0.12 ± 0.03	0.47 ± 0.01	0.56 ± 0.02
Somatic Cell Score	3.95	0.14 ± 0.03	0.36 ± 0.01	0.08 ± 0.01

[1]Sum of the additive genetic, permanent and temporary environmental variances.
[2]Ratio of the flock-test-day variance and the sum of the additive genetic, permanent environment, temporary environment and flock-test-day variances.

Heritability estimates for milk yield and milk composition traits were low and varied between 0.09 and 0.14. Standard errors of the heritability estimates were between 0.02 and 0.03. The heritability estimate for SCS was 0.14 with a standard error of 0.03.

The proportion of variation explained by FTD is large for all milk production traits; in particular, this proportion is larger than 0.50 for yield traits. Unlike the milk production traits, FTD does not have a big effect on SCS (0.08).

Repeatability estimates for all milk production traits and SCS ranged between 0.29 and 0.47 and the standard errors were always around 0.01. The lowest estimate was found for fat percentage.

Estimates of genetic and phenotypic correlations are in Table 2.3. The standard errors of the estimated genetic correlations ranged from 0.02 to 0.18, and for the phenotypic correlations, the standard errors were around 0.01. No estimates of correlations are reported between milk production and fat and protein yields. In these cases, the analysis did not converge, probably because the estimates were very close to unity. The genetic correlation between fat and protein yields was strong and positive (0.95). The genetic and phenotypic correlations between milk yield and fat content were equal to 0.19 and to -0.13, whereas the genetic and phenotypic correlations between milk yield and protein content were -0.04 and -0.23, respectively. The genetic and phenotypic correlations between fat and protein content were 0.74 and 0.53, respectively.

Table 2.3 Genetic (above the diagonal) and phenotypic (below the diagonal) correlations (± s.e.[1]) among test-day variables.

	MY (g)	Fat %	FY (g)	Protein %	PY (g)	SCS
MY (g)		0.19±0.18	-	-0.04±0.18	-	0.23±0.16
Fat %	-0.13		0.45±0.15[2]	0.74±0.09[2]	0.33±0.17	0.16±0.16
FY (g)	-	0.27		0.14±0.16	0.95±0.02[2]	0.31±0.15[2]
Protein %	-0.23	0.53	-0.04		0.19±0.17	0.24±0.14
PY (g)	-	-0.01	0.88	0.02		0.31±0.16
SCS	-0.12	0.14	-0.05	0.25	-0.05	

MY: milk yield; FY: fat yield; PY: protein yield; SCS: somatic cell score.
[1]For phenotypic correlations, the s.e. are <=0.01.
[2]These correlation estimates are significantly different from 0 (P<0.05).

Estimated genetic correlations between SCS and milk production traits were all positive. The estimates ranged from 0.16 to 0.31. The standard errors for the genetic correlations were high and ranged from 0.14 to 0.16. Phenotypic correlations of SCS with milk, fat and protein yields were negative (-0.12, -0.05 and -0.05), but positive with fat and protein contents (0.14 and 0.25).

2.4 Discussion

The means for fat and protein percentages were 6.80 and 5.48%. Cappio-Borlino et al. (1997) reported values of 6.84 for fat and 5.07 for protein in the same breed. The mean SCC was 1,484 (x 10^3 cells/mL) and similar to the value of 1,501 reported by Gonzalo et al. (1994). The mean SCS was higher than the value of 3.34 obtained by Barillet et al. (2001) in the Lacaune breed and the 3.80 found by Serrano et al. (2003) in the Manchega breed, using a lactation mean. However, this value is in the range reported in literature (from 5.26 to 12.1) for test-day models (i.e., El-Saied et al., 1998; Othmane et al., 2002).

The coefficients of variation calculated for milk production traits were in agreement with the coefficients of variation found in other studies (Baro et al., 1994; El-Saied et al., 1998; Hamann et al., 2004). Coefficients of variation of 50% for fat yield, 47% for protein yield, 25% for fat percentage and 19% for protein percentage have been calculated, based on the results reported by Hamann et al. (2004).

The coefficient of variation for SCC obtained in this study was consistent with the value of 238.27% reported by Baro et al. (1994). Such a high value for the coefficient of variation is due to the skewed distribution of SCC. The coefficient of

variation for SCS was 31% and similar to the one reported by Baro et al. (1994) in Churra sheep (28%) but lower than the value of 53% calculated, based on the results reported by Hamann et al. (2004) in East Friesian sheep. Instead of calculating the coefficient of variation for SCS, it is more appropriate to calculate the coefficient of variation for the log-transformed SCC, i.e. without adding the constant of 3 (Ali and Shook, 1980). The coefficient of variation for the log-transformed SCC is 56%, which is in the same order as the coefficients of variation for milk, fat and protein yields.

The heritability estimate for test-day milk yield was lower than those reported for other sheep breeds, which are between 0.15 and 0.24 (El-Saied et al., 1998; Barillet et al., 2001; Othmane at al., 2002). In the literature, heritabilities for fat and protein percentage estimated with test-day models range from 0.06 to 0.39 (i.e., El-Saied et al., 1998; Barillet et al., 2001; Othmane at al., 2002). Hamann et al. (2004) reported heritability estimates of 0.15 for both fat and protein yield which are similar to the estimates found in the present study.

The heritability estimate for SCS falls within the range reported in the literature. Results based on repeatability test-day models for SCS, indicated heritability estimates ranging from 0.04 for the Churra breed (Baro et al., 1994) to 0.16 for the East Friesian breed (Hamann et al., 2004). Other studies reported higher heritability estimates for the average SCS during lactation, from 0.11 to 0.18 (Mavrogenis et al., 1999; Barillet et al., 2001; Rupp et al., 2001). Based on our estimated heritability and repeatability, we expect to find a heritability for the average SCS of 5 observations equal to 0.29 (Falconer and Mackay, 1996).

The low heritability estimates for milk production traits in the present study could be due to parentage errors (Van Vleck, 1970). In the typical Sicilian semi-extensive system, it is common practice to have a number of active rams in a flock for unrecorded natural mating from March until December. Therefore, it is often not known with certainty which ram is the sire of an animal. It was hypothesized that the pedigree is more accurate on the female side than on the male side. To test if there were any differences, two analyses were performed, one in which all the sires were assumed to be unknown and one in which all the dams were assumed to be unknown. However, we did not find any evidence for the fact that heritability estimates were affected by pedigree errors, as the two analyses gave very similar heritability estimates which did not differ from the results reported in Table 2.2. In addition, pedigree errors would also have affected the heritability estimate for SCS. However, our heritability estimate for SCS is relatively high, which conflicts with the hypothesis that estimates were lower due to pedigree errors.

Parameter estimates could also have been influenced by genetic differences between flocks, due to the lack of genetic connections between Valle del Belice flocks. The genetic exchange between flocks is indeed limited; if farmers sell ewes to other producers, this usually occurs after the first lactation (Finocchiaro et al., 2005). Limited genetic links between flocks might hinder the separation of the genetic effects from flock effects. These possible genetic differences can be accounted for by using genetic groups in the model. Therefore, an analysis was carried out in which the base animals within each flock were assigned to different genetic groups. However, this model did not have a big effect on the estimated genetic parameters.

Table 2.2 shows that FTD effects explain a large proportion of the variation for milk production traits. Management of the Valle del Belice breed is indeed characterized by the enormous variability. Part of this variability is due to the fact that most of the farmers milk ewes by hand, but some of the farms use a milking machine. Furthermore, the lambing system is different from the one adopted in other Mediterranean regions (e.g., Carta et al., 1995; Ligda et al., 2000). The lambing season of the Valle del Belice breed is all year long, starting in July and finishing in the following June, with few lambings in May and June (Finocchiaro et al., 2005). The primiparous ewes usually give birth between December and March. Moreover, sheep are fed natural pastures and fodder crops; supplementation, consisting of hay and sometimes concentrates, is occasionally supplied, for example at the end of gestation (Cappio-Borlino et al., 1997). The grazing possibilities, the chemical and nutritional composition of the feed, change annually and also differ between areas. It is interesting to highlight that, unlike the milk yield traits, FTD does not have a big effect on SCS (0.08). This result might be due to the fact that with production traits, FTD affects all ewes. Hence the effects of the flock means on that test-day are large. But with SCS, we are probably looking at just a few high SCC ewes each time, and consequently the FTD effects will remain small.

Repeatability estimates for milk composition traits were moderate and comparable with those reported for dairy ewes (i.e., El-Saied et al., 1998; Othmane et al., 2002; Serrano et al., 2003). The repeatability for SCS (0.36) was consistent with those reported by El-Saied et al. (1998) and Othmane et al. (2002) for the Churra breed (0.38 and 0.34, respectively) but higher than the ones reported by Serrano et al. (2003) for the Manchega breed (0.22) and by Hamann et al. (2004) for the East Friesian breed (0.23).

At present, no genetic and phenotypic correlations between milk and fat yield and between milk and protein yield could be estimated. Correlations estimated using unadjusted data were 0.90 between milk and fat yield and 0.96 between milk and

protein yield. Sanna et al. (1997) reported genetic and phenotypic correlations equal to 0.89 and 0.93 between milk and fat yields and 0.94 and 0.97 between milk and protein yields. The genetic correlation between fat and protein yields was high and positive, indicating a strong association between these traits. This estimate was higher than the value of 0.68 reported by Hamann et al. (2004) and similar to the 0.93 reported by Sanna et al. (1997). Different genetic correlations from those obtained in this study were reported by Sanna et al. (1997) and by Othmane et al. (2002) between milk yield and fat content and between milk yield and protein content, whereas the phenotypic correlations between these traits obtained in the current study (-0.13 and -0.23) are similar to those reported by Sanna et al. (1997). The genetic and phenotypic correlations between fat and protein content were consistent with those reported by Sanna et al. (1997) and by Othmane et al. (2002). Hamann et al. (2004) reported higher genetic correlations between fat yield and fat content (0.53) and between protein yield and protein content (0.33) than those obtained in this study.

Estimated genetic correlations between SCS and milk production traits were all positive, indicating that selection for increased milk yield or fat and protein content will lead to higher SCS. The genetic correlations between production traits and SCS in cattle (for a review see Mrode and Swanson, 1996) resulted mostly in unfavorable genetic correlations (i.e., high milk associated with high level of SCC). In dairy sheep, estimated genetic correlations between milk yield and SCS are very different, ranging from antagonistic, i.e., from 0.04 to 0.18 (Barillet et al., 2001; Rupp et al., 2003), to favorable, i.e., from -0.15 to -0.37 (Baro et al., 1994; El-Saied et al., 1998 and 1999). The phenotypic correlation between milk yield and SCS obtained in this study falls in the range (between -0.15 and -0.05) reported in the literature (i.e., Baro et al., 1994; El-Saied et al., 1998; Othmane et al., 2002). A genetic correlation equal to 0.31 has been estimated between SCS and both fat and protein yields. These estimates are very different from those reported by Hamann et al. (2004) in East Friesian sheep (-0.04 and 0.06, respectively). Positive and low to moderate genetic correlations were estimated between SCS and fat content (0.16) and between SCS and protein content (0.24). These results were usually higher than those obtained in other studies (i.e., El-Saied et al., 1998; Othmane et al., 2002; Hamann et al., 2004); although Baro et al. (1994) reported a higher genetic correlation between SCS and protein content (0.37). Therefore, these results suggest that an increase in somatic cells occurs with an increase of fat and protein contents.

2.5 Conclusions

Heritability estimates for milk production traits in Sicilian Valle del Belice sheep are from 0.09 to 0.14. These values are lower than those reported for other sheep breeds. The heritability for SCS is 0.14 and falls within the range reported in other studies. There is a substantial effect of flock-test-day on milk production traits. However, the effect of FTD on SCS is limited. The analyses have also shown that SCS is genetically positively correlated to milk, fat and protein yields and contents. Therefore, selection for increased milk production will also increase SCS. However, correlations are not extreme, so simultaneous improvement for milk yield and SCS seems possible.

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3

Effect of somatic cell count level on functional longevity in Valle del Belice dairy sheep assessed using survival analysis

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Abstract

The objectives of this study were to evaluate the effect of somatic cell count (SCC) on functional longevity and to estimate the heritability of functional longevity using survival analysis in Valle del Belice dairy sheep. A total of 4,880 lactations of 2,190 ewes from 11 flocks were used. In this study, SCC was considered as an indication of sub-clinical mastitis. In case of clinical cases, identified by the technicians at milking time, test-day weights and milk samples of those ewes were not considered. Somatic cells were analyzed as counts, without any transformation, and were grouped in 3 classes based on the observed SCC maximum (mxSCC). The mxSCC classes, expressed as 10^3 cells/mL, were classified as 1 if mxSCC $\leq$ 500, 2 if 500 < mxSCC < 1000, and 3 if mxSCC $\geq$ 1000. An increase in SCC was associated with an increased hazard of being culled. Ewes in the highest class of SCC on a test-day had a 20% higher hazard of being culled than those in the lowest class. Therefore, SCC played a role in culling decisions of Valle del Belice dairy sheep farmers. The heritability estimate for functional longevity was 7% on the logarithmic scale and 11% on the real scale, indicating that selection for this trait is possible in sheep. The flock-year-season effect explained 19% of the variation on the logarithmic scale and 27% of the variation on the real scale.

Key words: somatic cell count, longevity, survival analysis, dairy sheep

3.1 Introduction

Intramammary infections are the primary cause of mastitis in dairy ewes and cows. Mastitis leads to economic losses, mainly arising from discarded milk, reduced milk production and quality, and increased health care costs both in dairy ewes (Leitner et al., 2004) and cows (e.g., Wellenger et al., 2002). Albenzio et al. (2002) reported a reduction in fat and casein content in ewes infected by mastitis. Almost all sheep milk is processed into cheese; thus, any change in casein content would have a substantial effect on the industrial value of the milk.

Legarra et al. (2007) reported that increased susceptibility to mastitis is one of the reasons for culling in sheep. However, little information is available about the relationship between longevity and both clinical and subclinical mastitis in sheep. Bergonier and Berthelot (2003) reported that annual incidence of clinical mastitis in sheep is generally less than 5%, whereas the prevalence of subclinical mastitis ranges from less than 10 to more than 50%. Barillet et al. (2001) reported a 5% frequency of culling for clinical mastitis and a 9.7% frequency for sub-clinical mastitis as predicted by SCC. In cattle, the incidence of mastitis has an important effect on culling decisions (e.g., Neerhof et al., 2000), particularly mastitis that occurs before the time of peak milk yield (Beaudeau et al., 1994). Antagonistic genetic correlations for longevity with mastitis resistance, ranging from 0.22 to 0.53, were found in dairy cows (Nielsen and Pedersen, 1995; Mrode et al., 2000; Roxström and Strandberg, 2002).

Mastitis causes an increase in SCC in small ruminants (Zeng et al., 1997; Leitner et al., 2004) and cattle (e.g., Heringstad et al., 2006). Moreover, mastitis data are difficult and expensive to collect, whereas SCC is currently recorded in several milk recording schemes in both dairy sheep (Astruc et al., 2004) and cattle (Boettcher, 2005). Therefore, SCC is promoted as an indirect method of predicting mammary infections and as a selection criterion to improve mastitis resistance (Heringstad et al., 2000; Barillet, 2007). However, Legarra et al. (2007) considered the measure of SCC as an indicator of subclinical mastitis. Whereas clinical mastitis is generally identified by evident signs, subclinical mastitis is usually inferred from SCC (Bergonier and Berthelot, 2003).

In dairy cattle, the effect of SCC on culling at the phenotypic level was first assessed by Beaudeau et al. (1995). Previous studies, using survival analysis, reported that higher concentrations of SCC were associated with higher rates of culling (Samoré et al., 2003; Caraviello et al., 2005). Antagonistic genetic correlations between SCC and longevity, ranging from 0.16 to 0.36, have been reported for dairy cows (Nielsen and Pedersen, 1995; Mrode et al., 2000; Roxström and Strandberg, 2002),

indicating that elevated SCC is associated with reduced longevity. However, information regarding the relationship between SCC and functional longevity is lacking in sheep. Legarra et al. (2007) suggested that a much more detailed study on culling policies and relationships between SCS, SCC, mastitis and survival in dairy sheep is needed.

Longevity corrected for milk production level, functional longevity, is an approximate measure for involuntary culling (Dekkers, 1993). At present, only a few estimates of heritability for longevity are available in dairy sheep (Conington et al., 2001; El-Saied et al., 2005). The objectives of this study were to evaluate the effect of SCC as an indirect measure of subclinical mastitis on functional longevity, and to estimate the heritability of functional longevity in Valle del Belice dairy ewes.

3.2 Material and methods

The original data set consisted of 6,530 lactations of 3,219 ewes. Data for SCC were collected at approximately 1-mo intervals by the University of Palermo (Italy) in 17 Valle del Belice flocks between 1998 and 2006; SCC was measured only when ewes were free of clinical mastitis. The Valle del Belice breed is the most productive autochthonous breed reared in Sicily. At milking time, cases of clinical mastitis were identified by the technicians and test-day weights and milk samples of those ewes were not considered. Clinical mastitis was reported for the evident signs of udder inflammation, or abnormal milk, or both. Most cases of mastitis were confirmed by a veterinarian. Records were excluded when SCC information was missing, when ewes had missing sire identification or ewes were sired by rams with fewer than 4 female offspring. Moreover, records were left out when the age at first lambing was not in the range of 10 to 30 months and when they were from flocks with observations for a period of less than 2 years. After editing, the data set consisted of 4,880 lactation records from 2,190 ewes reared in 11 flocks. The average number of SCC test-days was 3.5 per lactation.

The response variable was the productive life defined on a lactation basis, either from one lambing to the next or to culling, whichever came first, in accordance with Roxström et al. (2003). In this analysis, the overall longevity for a given ewe was partitioned into lactation periods; consequently, all records began with a lambing. In cases where ewes were not culled during a specific lactation period, records were treated as right censored. Ewes were considered culled, and therefore records were considered uncensored, if the last test-day reported was at least 6 months before the end of the observation period for each flock. The 6-

month period was chosen by considering the intervals between lambings and the possibility that a ewe was in a dry period.

The analysis was performed with a survival analysis (Ducrocq and Casella, 1996) using the Survival Kit v.3.0 set of programs (Ducrocq and Sölkner, 1998a). A Weibull model, less computationally demanding compared to the Cox model, was used. The Weibull distribution assumption was checked by plotting $\ln(-\ln S(t))$ against $\ln(t)$, where *S(t)* was the Kaplan-Meier estimate of the survivor function, *ln* was natural logarithm, and *t* was the number of days from lambing within lactation.

The following Weibull model was used:

$$h_{ijlmnop}(t) = h_0(t)\exp\left[sl_i(t) + op_j + age_l + mk_m(t) + scc_n(t) + fys_o + s_p\right]$$

where:

$h_{ijlmnop}(t)$ was the hazard of culling for a given ewe at time t, being *t* days from lambing within a lactation;

$h_0(t)$ was the Weibull baseline hazard function, with scale parameter λ, and shape parameter ρ;

$sl_i(t)$ was the time-dependent fixed effect of the *i*th stage of lactation. Five classes were defined as 1 when 0 < DIM ≤ 60, 2 when 60 < DIM ≤ 120, 3 when 120 < DIM ≤ 180, 4 when 180 < DIM ≤ 250, 5 when DIM > 250. The stage of lactation effect was included to account for changes in the culling hazard within lactation;

op_j was the time-independent fixed effect of the *j*th parity, with *j* = 1, …, 5;

age_l was the time-independent fixed effect of the *l*th age at first lambing class, where *l* = 1 when first lambing occurred at 10 to 18 months of age, 2 at 19 to 23 months of age, and 3 at 24 to 30 months of age. When the information about the age at first lambing was missed, such as when ewes were recorded from a second or later lactation, the age at first lambing was approximated by considering a fixed interval between parities equal to 365 days;

$mk_m(t)$ was the time-dependent fixed effect of the *m*th class for average daily milk production, expressed as milk deviations (milk_dev) from the mean and standardized by the corresponding standard deviation, within stage of lactation and flock. The milk deviations were assumed to be piecewise constant from the beginning to the end of a given stage of lactation. Three classes were considered, *m* = 1 if milk_dev < −1, *m* = 2 if −1 < milk_dev ≤ +1, and *m* = 3, milk_dev > +1;

$scc_n(t)$ was the time-dependent fixed effect of the *n*th class, based on the observed SCC maximum (mxSCC) within a stage of lactation; it was assumed to be piecewise constant from the beginning to the end of a given stage of lactation. The mxSCC

classes, expressed as 10^3 cells/mL, were classified as 1 if mxSCC ≤ 500 (with 3,690 records), 2 if 500 < mxSCC < 1000 (with 1,409 records), and 3 if mxSCC ≥ 1000 (with 2,867 records). Thresholds for SCC classes were chosen according to Bergonier et al. (1994) and based on the evidence provided by other authors that healthy sheep normally have higher SCC than cows (e.g., Fthenakis et al., 1991);

fys_o was the time-independent random effect of the *o*th flock-year-season of the lambing subclass, with *o* = 1, …, 166 from up to 11 flocks in 9 years of lambing, considering three lambing seasons. The lambing season was equal to 1 when lambing occurred from August to November, equal to 2 from December to March, and equal to 3 from April to July (Portolano et al., 2007). A *fys* class was included in the analysis if there were at least four records within it. The *fys* effects were assumed to follow a log-gamma distribution with parameter γ;

s_p was the time-independent random effect of sire (*p* = 1, …, 168), assumed to be distributed as a multivariate normal with mean vector 0 and covariance matrix $\mathbf{A}\sigma_S^2$, where **A** was the additive relationship matrix among sires. A sire model was used. Heritability was estimated as (Ducrocq and Casella, 1996):

$$\hat{h}^2_{\log} = \frac{4\sigma_S^2}{\psi(\gamma) + \sigma_S^2 + \frac{\pi^2}{6}},$$

where $\psi(\gamma)$ is the trigamma function evaluated at the estimated marginal posterior mode of the variance of the flock-year-season effect and π is the pi constant. The effective heritability was estimated as (Yazdi et al., 2002):

$$\hat{h}^2_{eff} = \frac{4\sigma_S^2}{\psi(\gamma) + \sigma_S^2 + 1}.$$

Standard error for the effective heritability was approximated (Roff, 1997; eqn 2.28). By replacing the numerator $4\sigma_S^2$ with $\psi(\gamma)$, we estimated the proportion of the total variation explained by the flock-year-season effect, on both logarithmic and real scales.

3.3 Results

Longevity for a given ewe was partitioned into lactations and, by consequence, about 76.4% of the records were right censored. The average lactation length, considering the entire dataset (culled and not culled animals), was 171 ± 72 d; whereas the lactation length of animals which were not culled was 250 ± 35 d. The

daily average of milk yield was 1,310 g, whereas the average test-day SCC was 1,932 x 10^3 cells/mL.

The fitted model explained approximately 50% of the total variation in the response variable. The shape parameter ρ was equal to 1.33, indicating that as time increased within lactation, so did the hazard of culling. The plot used to check the Weibull distribution assumption, $\ln\left(-\ln S(t)\right)$ *vs.* $\ln(t)$, showed a straight line (results not shown), an indication that this assumption was valid.

Chi-square test approximations based on likelihood ratio test (LRT) were calculated for all the effects in the model (Table 3.1). The significance of each effect resulted from the corresponding p-value.

Table 3.1 Chi-square (χ^2) tests based on likelihood ratio tests for all effects in the model.

Effects in the model	[1]DF	χ^2	p-value
Stage of lactation	4	113.4	< 0.0001
Parity	4	48.4	< 0.0001
Age at first lambing	2	7.1	0.0282
Daily milk production	2	121.5	< 0.0001
Maximum SCC	1	10.2	0.0014
Flock-year-season	1	309.9	< 0.0001
Sire	1	31.8	< 0.0001

[1]DF: degrees of freedom; χ^2 is a Likelihood Ratio Test (LRT), i.e. the difference between the -2 log(likelihood) for the reduced model (without the effect tested) and the -2 log(likelihood) for full model.

Estimates of time-independent fixed effects indicated that ewes at second lambing were at a lower hazard of being culled than ewes at first lambing (Table 3.2). Relatively more culling in first parturition might be explained by culling for low production or culling for lambing difficulties, especially for ewes having the first lambing at an early age. Moreover, ewes at the fourth or greater lambing were at a higher hazard than ewes at the first lambing. The effect of age at first lambing resulted, in general, in an increase in culling rate with age.

Table 3.2 Mode ($\hat{b}$) and standard deviation ($\hat{\sigma}_{\hat{b}}$) of the marginal posterior distributions, hazard ratio ($\hat{h}$) based on posterior modes, and 95% Confidence Interval (CI) for $\hat{h}$ with lower and upper bounds for the time-independent fixed effects, estimated with the model including SCC.

Effect[1]	Classes	$\hat{b}$	$\hat{\sigma}_{\hat{b}}$	$\hat{h}$	95% CI for $\hat{h}$	
					Lower	Upper
Parity	1	0	—	1	—	—
	2	-0.216	0.093	0.805	0.672	0.966
	3	-0.095	0.107	0.909	0.738	1.121
	4	0.237	0.115	1.268	1.013	1. 588
	5	0.608	0.121	1.837	1.450	2.328
Age at first lambing	1	0	—	1	—	—
	2	0.107	0.083	1.113	0.945	1.311
	3	0.236	0.087	1.266	1.066	1.502

[1]parity = 1, and age at first lambing = 1 were used as reference levels.

Table 3.3 Mode ($\hat{b}$) and standard deviation ($\hat{\sigma}_{\hat{b}}$) of the marginal posterior distributions, hazard ratio ($\hat{h}$) based on posterior modes, and 95% Confidence Interval (CI) for $\hat{h}$ with lower and upper bounds for the time-dependent fixed effects, estimated with the model including SCC.

Effect[1]	Classes	$\hat{b}$	$\hat{\sigma}_{\hat{b}}$	$\hat{h}$	95% CI for $\hat{h}$	
					Lower	Upper
Stage of lactation	1	1.053	0.273	2.866	1.677	4.899
	2	1.361	0.175	3.901	2.768	5.497
	3	1.270	0.142	3.562	2.694	4.708
	4	0.876	0.126	2.400	1.874	3.074
	5	0	—	1	—	—
Daily milk production	1	0.925	0.116	2.523	2.008	3.169
	2	0.222	0.109	1.248	1.008	1.546
	3	0	—	1	—	—
Maximum SCC	1	0	—	1	—	—
	2	0.172	0.099	1.187	0.978	1.441
	3	0.214	0.070	1.239	1.079	1.421

[1]stage of lactation = 5, daily milk production = 3, and maximum SCC = 1 were used as reference levels.

Table 3.3 shows the estimates of time-dependent fixed effects. The estimates for the first to fourth classes of stage of lactation were significantly different from 1, indicating that ewes in these classes were at a higher hazard of being culled than those in the fifth class, the reference level. Ewes in the first and second classes of milk production were at a higher hazard of being culled than those in the third (reference) class with greatest milk yields. Regarding SCC, ewes in the second and third classes were at a higher hazard of being culled than those in the first class with the least SCC.

Table 3.4 Mean (± SD) and mode of the random effects, heritabilities for the productive life on logarithmic ($\hat{h}^2_{\log}$) and real scales ($\hat{h}^2_{eff}$), standard error (S.E.) of $\hat{h}^2_{eff}$, and proportion of variation explained by flock-year-season on logarithmic ($Pfys_{\log}$) and real scales ($Pfys_{eff}$).

Mean (± SD) σ^2_{sire}	0.047 (± 0.026)
Mean (± SD) σ^2_{fys}	3.193 (±0.627)
Mode σ^2_{sire}	0.039
Mode σ^2_{fys}	3.041
$\hat{h}^2_{\log}$	0.07
$\hat{h}^2_{eff}$	0.11
S.E.($\hat{h}^2_{eff}$)	0.025
$Pfys_{\log}$	0.19
$Pfys_{eff}$	0.27

Table 3.4 shows the estimates for the time-independent random effects, heritabilities for productive life, and proportion of variation explained by the flock-year-season effect obtained with the Weibull model. The heritability of productive life was 0.07 on the logarithmic scale and 0.11 on the real scale. The proportion of variation in productive life explained by the flock-year-season effect was 0.19 on the logarithmic scale and 0.27 on the real scale.

3.4 Discussion

Prognostic factors - particularly SCC, which serve as an indirect measure of subclinical mastitis - affected productive life in Valle del Belice ewes. To our knowledge, this is the first such finding in sheep, using survival analysis. Therefore, results were mostly compared with those reported for dairy cattle. A lactation basis

approach was chosen because it better suited our data, which were collected on farms during different periods. Roxström et al. (2003) did not report major differences between longevity analysed on a lactation basis and longevity based on the entire length of life.

The effect of age at first lambing resulted, overall, in an increase in culling rate with age. It is not convenient for a farmer to keep animals that are going to lamb for the first time at an advanced age. This result was in agreement with the results reported by Samoré et al. (2003) in dairy cattle. However, Chirinos et al. (2007) reported that age at first calving did not affect the hazard of culling in the Spanish Holstein-Friesian cattle population.

The effect of stage of lactation was included in the model because it allowed for better modeling of the baseline hazard and has an important factor affecting culling. Ewes in middle lactation (i.e., 60-180 days) had a higher hazard of being culled compared to those in late lactation. When referring only to culling because of mastitis, a review by Bergonier et al. (2003) showed that in sheep, the majority of cases of mastitis occur during the first third of lactation. In dairy cattle Beaudeau et al. (1995) and Roxström et al. (2003) reported an increased hazard of culling in early lactation.

The estimates for the classes of daily milk production showed that ewes with a level of production below the flock average had a higher hazard of being culled, in agreement with research results in cattle (e.g., Vukasinovic et al., 2001; Chirinos et al., 2007).

Ewes with a maximum of SCC between 500 and 1000 or ≥ 1000 × 10^3 cells/mL in a stage of lactation had a slightly higher hazard of being culled than ewes with lesser maximums. These estimates were in the same direction as those reported by Beaudeau et al. (1995) in dairy cows, with a range of 1.7 from the highest to lowest class for SCC, whereas they were much lower than those reported by Samoré et al. (2003). Samoré et al. (2003) considered SCS instead of SCC, reporting a 3-fold higher rate of culling for cows with test-days in the highest classes of SCS. However, using SCC or SCS is equivalent when classes are used. The transformation of SCC into SCS, therefore, would not have changed these classes because the particular transformation that is used to go from SCC to SCS is a one-to-one function. The risk associated with SCC level estimated in this study was less and might be explained by the fact that most Valle del Belice farmers do not directly use SCC information to make culling decisions for animals. In sheep, the current milk payment system of most countries is based only on milk yield and not on SCC level, which is different from the cattle industry. However, Valle del Belice sheep milk is mainly used for producing raw-milk traditional cheeses (Pecorino and Vastedda del Belice cheeses)

either by farms, small local dairies, or cheese industries working at the regional level. Therefore, an indirect effect of SCC on culling can be considered because SCC has a remarkable influence on the bulk milk composition and lactodynamographic parameters of Valle del Belice sheep (Giaccone et al., 2005). Moreover, the genetic correlations between milk production traits (milk yield, fat and protein yields and contents) and SCS level in the Valle del Belice sheep are positive, ranging from 0.16 to 0.31 (Riggio et al., 2007). However, Bufano et al. (1994) showed that high SCC (> 1 million/mL) do occur in normal sheep milk, especially toward the end of lactation, whereas Bergonier et al. (2003) showed that nonpathological factors are responsible for variations of SCC in ewe milk between 40×10^3 and 100×10^3 cells/mL.

Considering SCC as an indicator of subclinical mastitis, these results may be compared with studies analyzing the rate of culling directly associated with mastitis incidence. Beaudeau et al. (1995) found that udder health disorders were always highly related to an increase in culling rate in dairy cows. Legarra et al. (2007), in a study to derive the economic value of SCS in dairy sheep, reported that SCS had an effect on culling decision. In this paper the economic weight of culling was considered indirectly based on the genetic correlations between SCS level and the culling due to subclinical mastitis. A genetic increase of one unit of SCS increases the trait 'culled by mastitis' in 0.12 times the genetic correlation.

In the present study, the flock-year-season effect was an important factor explaining the observed variation in productive life. This result was in agreement with the results from other studies on Valle del Belice sheep (Portolano et al., 2007; Riggio et al., 2007), confirming the importance of this factor. Milking practices might be an important factor in influencing udder health. In Sicily, only a few farmers use a milking machine; most farmers milk ewes by hand. It is not common for farmers to wash ewes' udders or their hands before milking. Moreover, antibiotic dry therapy is not implemented on all farms. This variability is reflected in different hygiene conditions among farms, which can influence milk quality.

Literature concerning sheep reports heritability estimates for functional longevity somewhat lower than those presented in this study. El-Saied et al. (2005) reported a heritability of 0.05 for functional longevity in the Churra breed. Similar estimates of heritability for longevity, measured as days or years in the flock, were reported in the literature for meat breeds (0.06 for Australian Dorset sheep, Brash et al., 1994; 0.08 for Scottish Blackface, Conington et al., 2001). The heritability estimated for functional longevity in this study was higher than estimates reported by Chirinos et al. (2007) in cattle, ranging between 0.05 and 0.07. Whereas Neerhof et

al. (2000) and Ducrocq and Solkner (1998b) reported heritabilities around 0.05 for the logarithmic scale and 0.22 for the real scale.

3.5 Conclusions

An increase in SCC as an indicator of subclinical mastitis was associated with an increase in rate of culling. Therefore, elevated SCC arising from subclinical mastitis played an indirect role in the culling decisions of Valle del Belice dairy sheep farmers although, at present, they do not select directly to reduce SCC. The proportion of additive genetic variation estimated for functional longevity in Valle del Belice ewes indicates that it may be possible to improve productive life by genetic selection. The consistent flock-year-season effect estimated confirms the high variability in management of the Valle del Belice breed.

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4

Genetic parameters for somatic cell score according to udder infection status in Valle del Belice dairy sheep and impact of imperfect diagnosis of infection

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Abstract

Somatic cell score (SCS) has been promoted as a selection criterion to improve mastitis resistance. However, SCS from healthy and infected animals may be considered as separate traits. Moreover, imperfect sensitivity and specificity could influence animals' classification and impact on estimated variance components. This study was aimed at: (1) estimating the heritability of bacteria negative SCS, bacteria positive SCS, and infection status, (2) estimating phenotypic and genetic correlations between bacteria negative and bacteria positive SCS, and the genetic correlation between bacteria negative SCS and infection status, and (3) evaluating the impact of imperfect diagnosis of infection on variance component estimates. Data on SCS and udder infection status for 1,120 ewes were collected from four Valle del Belice flocks. The pedigree file included 1,603 animals. The SCS dataset was split according to whether animals were infected or not at the time of sampling. A repeatability test-day animal model was used to estimate genetic parameters for SCS traits and the heritability of infection status. The genetic correlation between bacteria negative SCS and infection status was estimated using an MCMC threshold model, implemented by Gibbs Sampling. The heritability was 0.10 for bacteria negative SCS, 0.03 for bacteria positive SCS, and 0.09 for infection status, on the liability scale. The genetic correlation between bacteria negative and bacteria positive SCS was 0.62, suggesting that they may be genetically different traits. The genetic correlation between bacteria negative SCS and infection status was 0.51. We demonstrate that imperfect diagnosis of infection leads to underestimation of differences between bacteria negative and bacteria positive SCS, and we derive formulae to predict impacts on estimated genetic parameters. The results suggest that bacteria negative and bacteria positive SCS are genetically different traits. A positive genetic correlation between bacteria negative SCS and liability to infection was found, suggesting that the approach of selecting animals for decreased SCS should help to reduce mastitis prevalence. However, the results show that imperfect diagnosis of infection has an impact on estimated genetic parameters, which may reduce the efficiency of selection strategies aiming at distinguishing between bacteria negative and bacteria positive SCS.

Key words: somatic cell count, infection status, genetic parameter, imperfect diagnosis, dairy sheep

4.1 Introduction

Somatic cell count (SCC), and therefore somatic cell score (SCS) have been widely promoted as an indirect method of predicting mammary infections (Ødegård et al., 2003) and as a selection criterion to improve mastitis resistance (Gonzalo et al., 2002). It has been demonstrated that mastitis is associated with an increase in SCC in small ruminants (Bergonier et al., 2003; Leitner et al., 2004) and cattle (Heringstad et al., 2006; Olde Riekerink et al., 2007). Hence, milk with an elevated SCC is usually considered an indication of the occurrence of infection in the udder; and selection for decreased SCC could lead to reduced susceptibility to mastitis (Mrode and Swanson, 1996).

However, one difficulty in using SCC to find animals most resistant to mastitis is that factors known to influence SCC have different magnitude in healthy and infected animals (Detilleux and Leroy, 2000), and SCC in healthy and in infected animals may even be considered as different traits. Indeed, it has been shown that cells in the milk from a healthy udder are mainly mammary gland epithelium and drain canal cells; whereas polymorphonuclear leukocytes (PMN) are the major cell population during early inflammation, playing a protective role against infectious diseases in the mammary gland (Kherli and Shuster, 1994; Persson-Waller et al., 1997). Therefore, in principle SCC from healthy and infected animals should be analyzed separately. However, because the intramammary infection status is generally unknown, one model is usually applied indifferently to SCC obtained from all animals, irrespective of whether they are infected or not. Test-day SCC may, therefore, be regarded as a mixture of observations from animals with unknown health status (Ødegård et al., 2003). We are in the fortunate position of having a dataset of SCC in dairy sheep for which bacteriological data are also available, indicating whether an animal was infected at the time of sampling. Therefore, instead of using mixture models to determine the infection status (Ødegård et al., 2003; Gianola et al., 2004), we were able to analyze SCC, and therefore SCS, separately in apparently healthy and infected animals.

Fundamental to any diagnostic test are the concepts of sensitivity and specificity. Sensitivity (Se) measures the proportion of actual positives (i.e., diseased animals) which are correctly identified as such by the diagnostic test; whereas specificity (Sp) measures the proportion of negatives (i.e., healthy animals) which are correctly identified by the diagnostic test. If the diagnostic test is perfect, both *Se* and *Sp* are equal to unity. However, if the diagnostic test is imperfect, i.e., *Se* and *Sp* are less than unity, *Se* and *Sp* will influence classification of animals and potentially impact on estimable variance components and inferences drawn from

the data. *Se* and *Sp* for the bacteriological assessments are unknown in our dataset, but it is likely that they were less than unity due to intermittent shedding of bacteria after infection and the possibility of contamination during sampling.
The aims of this study, therefore, were: (1) to estimate the heritability of SCS, according to whether the animals were healthy or infected, as assessed by our bacteriological data, along with the heritability of the infection status; (2) to estimate the phenotypic and genetic correlations between the bacteria negative SCS (i.e., apparently healthy animals) and the bacteria positive SCS (i.e., infected animals), and the genetic correlation between the bacteria negative SCS and the infection status; and (3) to evaluate the impact of imperfect diagnostic *Se* and *Sp* on variance component estimates for the traits of interest.

4.2 Material and methods

The data consisted of 9,306 test-day records from 2,058 lactations of 1,125 ewes. Data for SCC were collected at approximately 1-month intervals, following an A4 recording scheme (ICAR, 2003), by the University of Palermo in four Valle del Belice flocks between 2004 and 2007. At the same time, milk samples were collected aseptically from each animal for bacteriological analyses, which were performed by conventional techniques, on 5% sheep blood agar plates, incubated at 37°C, and examined after 10-24 h and 36-48 h incubation. The bacteriological colonies observed were mainly: *Staphylococcus aureus*, coagulase negative staphylococci, *Staphylococcus intermedius* and other staphylococci; *Streptococcus canis*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus agalactiae* and other streptococci; *Corynebacterium* spp., *Pasteurella* spp., and *Pseudomonas* spp. (Table 4.1).
Ewes were considered infected if more than five colony forming units (CFU) per 10 µl of milk of one species of bacteria were isolated, and the data used in this study were the apparent presence or absence of infection for each milk sample.
All test-day records used in the analysis were required to have information regarding SCC and bacteriological status. After editing, the data comprised 8,843 test-day records from 2,047 lactations of 1,120 ewes. The pedigree file included 1,603 animals. In addition to the 1,120 animals with records, 84 sires and 399 dams without phenotypes were included in the pedigree. On average, the sires served at least two of the four flocks under study and they had 13.33 daughters on average.

Table 4.1 Number of observations and frequencies for bacteria observed.

	Number of observations	Frequency (%)
Staphylococcus aureus	300	10.47
coagulase negative staphylococci	2316	80.81
Staphylococcus intermedius	36	1.26
Other staphylococci	20	0.70
Streptococcus canis	6	0.21
Streptococcus dysgalactiae	23	0.80
Streptococcus uberis	12	0.42
Streptococcus agalactiae	12	0.42
Other streptococci	84	2.93
Corynebacterium spp.	7	0.24
Pasteurella spp.	40	1.40
Pseudomonas spp.	10	0.34

For analyses investigating the properties of SCC in ewes with either positive or negative bacteriological status, we divided the data in two sub-datasets: one sub-dataset comprising test-day records with the presence of infection (bacteria positive) and the accompanying SCC information (2,866 test-day records from 1,263 lactations of 805 ewes), and the other one comprising test-day records with the absence of infection (bacteria negative) and the accompanying SCC information (5,977 test-day records from 1,805 lactations of 1,062 ewes). Because the dataset was divided by test-day records, the same animals could appear in both sub-datasets and they could even appear in both datasets in the same lactation. Of the 1,120 ewes from the original data, 744 were included in both sub-datasets.

The test-day traits analyzed as response variables were SCS and the infection status. SCS were obtained after log-transformation of test-day SCC, using a base 2 logarithmic function: $SCS = \log_2(SCC/100) + 3$ (Ali and Shook, 1980), in order to get an approximated normal distribution for this trait. An infection status trait was created, based on the presence/absence of pathogens, indicating whether ewes were infected (1) or apparently healthy (0) at each test-day.

Variance components and genetic parameters for SCS (whole dataset as well as bacteria negative and positive subsets) were estimated using ASReml (Gilmour et al., 2002).

The following repeatability test-day animal model as described by Riggio et al. (2007) was used to analyze the data:

$$y_{ijklmn} = \mu + FTD_i + YPS_j + P_k + LS_l + \beta_1 DIM_{ijklmn} + \beta_2 \exp\left(-0.05 * DIM_{ijklmn}\right) + A_m + PEm + PE_{km} + e_{ijklmn}$$

where y_{ijklmn} was the SCS test-day measurement; μ was the population mean; FTD_i was the random effect of flock by test-day interaction *i* (91 levels); YPS_j was the fixed effect of year by season of lambing interaction *j* (6 levels), where the season of lambing was coded as 1 if a ewe gave birth in the period January through June, otherwise it was coded as 2 (Riggio et al., 2007); P_k was the fixed effect of the parity (3 levels); LS_l was the fixed effect of litter size class *l* (2 levels, single or multiple born lambs); DIM_{ijklmn} and $\exp\left(-0.05 * DIM_{ijklmn}\right)$ were two covariates used to model the shape of lactation curves (Wilmink, 1987); A_m was the random additive genetic effect of the individual *m* (1,603 levels); PE_m was the general random permanent environmental effect of ewe *m* across lactations (1,120 levels); PE_{km} was the random permanent environmental effect on the individual *m* within parity class *k* (2,047 levels); e_{ijklmn} was the random residual effect. The same model was used for the analysis of the two sub-datasets.

Variance components and heritability for the infection status were first estimated using an animal linear model accounting for the same effects included in the model used for SCS. Then, a threshold animal model was fitted, assuming a probit link function.

Phenotypic and genetic correlations between SCS in the bacteria negative and positive subsets were estimated using bivariate analyses, fitting the same fixed and random effects as previously described. Given the data structure, i.e., non-contemporaneous bacteria negative and positive SCS observations for any individual, the environmental covariance between the two traits was assumed to be zero and not estimated when the genetic correlation was estimated. However, covariances were fitted for the additive genetic term and for the permanent environmental effects of the ewe both across and within lactations. To estimate an approximated phenotypic correlation, the data were restructured and reduced to adjacent pairs of bacteria negative and positive SCS data, i.e., the bacteria negative and positive SCS observations closest within one lactation were used. It should be noted that this approach does create a unique subset of SCS samples, as the bacteria negative SCS samples are from ewes either immediately prior to or post infection; conversely the bacteria positive SCS sample are from recovering or newly infected ewes. The same fixed effects, as previously described, were fitted but the

random effects model was simplified with (co)variance terms estimated only for additive genetic and residual effects.

The genetic correlation between the bacteria negative SCS and the infection status was estimated using TM (Threshold Model) program (available upon request to the author andres.legarra@toulouse.inra.fr), using a Bayesian analysis and performing numerical integration through the Gibbs sampler. The TM program does not handle covariates, so in this case the model was simplified and the two covariates of DIM were excluded. Flat priors were used both for fixed effects and variance components. A chain of 100,000 iterations was used, discarding the first 30,000 samples and saving a sample every 10 iterations. The mean of the estimated marginal posterior density has been used as point estimate of the genetic parameters of interest.

Genetic parameters for infection status, bacteria negative SCS, and bacteria positive SCS are potentially affected by imperfect *Sp* and *Se*, which were both implicitly assumed to be unity in the variance component estimation analyses. Additional file 1 shows the principles of the calculations used to show how imperfect *Se* and *Sp* can influence the interpretations of these data. Using the observed variance components, likely impacts of imperfect *Sp* and *Se* on estimated mastitis prevalence, predicted differences between SCS in bacteria negative and positive animals, and variance components were explored.

4.3 Results

Arithmetic means, standard deviations and range of SCC and SCS test-day traits are given in Table 4.2. The geometric mean SCC was 403 (x 10^3 cells/mL) for the whole data, 253 for the bacteria negative, and 1,082 for the bacteria positive.

Although ranges of SCC for uninfected and infected animals were similar, the arithmetic mean SCC for infected animals was approximately 3-fold higher than that for uninfected animals. This result suggests that although the distributions of bacteria negative and bacteria positive SCS partially overlap, they are substantially different as shown in Figure 4.1. The difference between bacteria positive and bacteria negative SCC may have been higher if SCC and infection status had been considered per udder half. However, we had only information at the animal level (summarizing the whole udder); therefore a dilution effect due to the mixing of milk with high SCC coming from infected glands and milk with low SCC from a healthy gland has to be considered.

Table 4.2 Descriptive statistics of SCC and SCS traits.

	Mean	SD	Range
Whole data SCC (x 10^3 cells/mL)	1,812	4,150	13 - 31,268
Whole data SCS	5.01	2.37	0.06 - 11.29
Bacteria negative SCC (x 10^3 cells/mL)	1,077	3,084	13 - 29,368
Bacteria negative SCS	4.34	2.06	0.06 - 11.20
Bacteria positive SCC (x 10^3 cells/mL)	3,346	5,462	16 - 31,268
Bacteria positive SCS	6.42	2.36	0.36 - 11.29

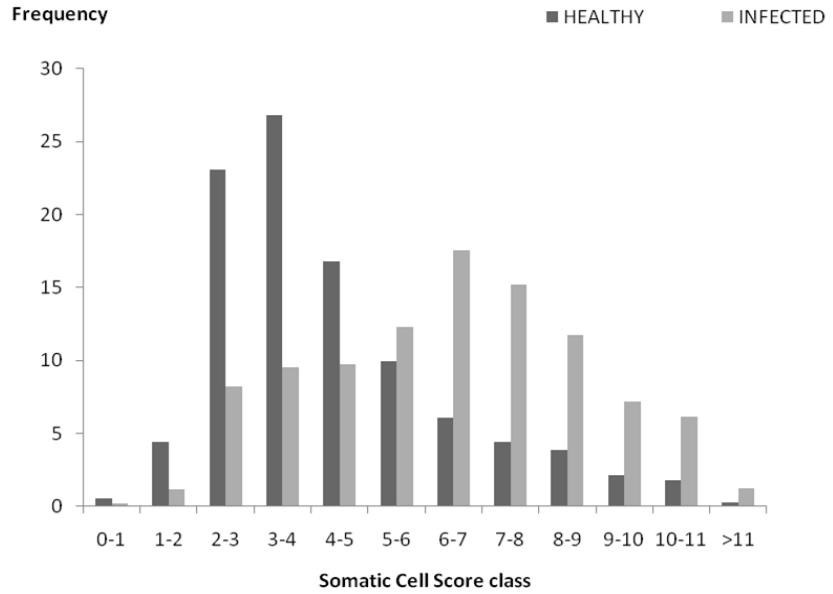

Figure 4.1 Distribution of bacteria negative (i.e. healthy) and bacteria positive (i.e. infected) SCS for the observed prevalence of bacteria positive milk samples (p' = 0.32).

Phenotypic, genetic, and environmental variances after adjustment for fixed effects, heritabilities and repeatabilities within and across lactations for SCS traits are given in Table 4.3. The heritability estimate for SCS was 0.09. However, estimates for bacteria negative and bacteria positive SCS were respectively 0.10 and 0.03. This difference could be due in part to the different sub-datasets (i.e., different animals and different number of records) used for the analysis. Therefore,

an analysis was carried out in which only the animals present in both sub-datasets were considered. However, this had little effect on the estimated heritabilities and did not change the interpretation of the results.

Table 4.3 Genetic parameters* for SCS traits.

	σ^2_p	σ^2_a	σ^2_e	$h^2 \pm se$	$r_{wit} \pm se$	$r_{acr} \pm se$
Whole data SCS	5.467	0.492	2.633	0.09±0.04	0.29±0.04	0.33±0.02
Bacteria negative SCS	2.225	0.223	1.188	0.10±0.06	0.21±0.04	0.30±0.03
Bacteria positive SCS	5.573	0.161	2.554	0.03±0.03	0.20±0.05	0.31±0.04

*Phenotypic (σ^2_p), genetic (σ^2_a), and environmental (σ^2_e) variances, heritability (h^2) and repeatability within (r_{wit}) and across (r_{acr}) lactations (± SE) for SCS traits

The observed phenotypic variance was 5.57 for infected animals and 2.23 for bacteria negative animals; whereas the observed genetic variance was 0.16 for infected animals and 0.22 for bacteria negative animals. Repeatability estimates within lactations ranged between 0.20 and 0.29, whereas repeatability estimates across lactations ranged between 0.30 and 0.33, and were higher than the within lactation values.

Table 4.4 shows the heritabilities of the infection status, estimated by considering the infection status both as a binary and continuous trait on the underlying scale, i.e., liability to infection, and the expected value on the underlying scale calculated from the binary scale using the approximation of Dempster and Lerner (1950). The heritability estimate obtained with the probit model was 0.09. As expected, the heritability estimate from the normal analysis was somewhat lower, and it can be seen that the assumption of the trait being continuous with normally distributed residuals is violated.

Table 4.4 Heritability for infection status with normal and probit analysis.

	Normal analysis* $h^2 \pm se$	Probit analysis** $h^2 \pm se$	Expected value† h^2
Infection status	0.05 ± 0.02	0.09 ± 0.04	0.09

*Treating the infection status as a continuous variable.
**Treating the infection status as a binary trait.
†Calculated with Dempster and Lerner's formula (1950).

However, the expected value on the underlying scale derived from the heritability estimate obtained with the normal analysis was the same as that from the binary trait analysis, confirming that the impact of departures from normality is predictable.

The phenotypic and genetic correlations between bacteria negative and bacteria positive SCS, and the genetic correlation between bacteria negative SCS and the infection status are presented in Table 4.5. The phenotypic correlation between bacteria negative and bacteria positive SCS was 0.19 (s.e. 0.02); whereas the genetic correlation was 0.62 (s.e. 0.12), indicating that whilst there is a moderate positive correlation between these traits it may be more appropriate to consider them as different traits. The genetic correlation between bacteria negative SCS and the infection status was 0.51, suggesting that animals with lower SCS, assessed when apparently not infected, are genetically less likely to be infected (across all time points). For completeness we also estimated the genetic correlation between SCS in bacteria positive animals and liability to infection. The estimated correlation was 0.81 but its biological interpretation is not obvious to us.

Table 4.5 Correlations* between SCS and infection status.

		Bacteria positive SCS	Infection status
Bacteria negative SCS	Genetic correlation:	0.62 ± 0.12	0.51
	Phenotypic correlation:	0.19 ± 0.02	***

*****Genetic and phenotypic correlations ($\pm$ SE**) between bacteria negative SCS and bacteria positive SCS, and genetic correlation between bacteria negative SCS and infection status

**SE is not reported for the correlation between bacteria negative SCS and infection status, as it was estimated using a Bayesian approach

***No attempt was made to estimate a phenotypic correlation between bacteria negative SCS and infection status

All analyses so far were done assuming the *Sp* = *Se* = 1. This may not be the case; although we have no data on the accuracy of the diagnoses, they are unlikely to be perfect. The impacts of imperfect diagnoses can be tabulated from formulae derived in Additional file 1. The impact of imperfect *Sp* or *Se* on the true prevalence, given the observed prevalence, is shown in Figure 4.2.

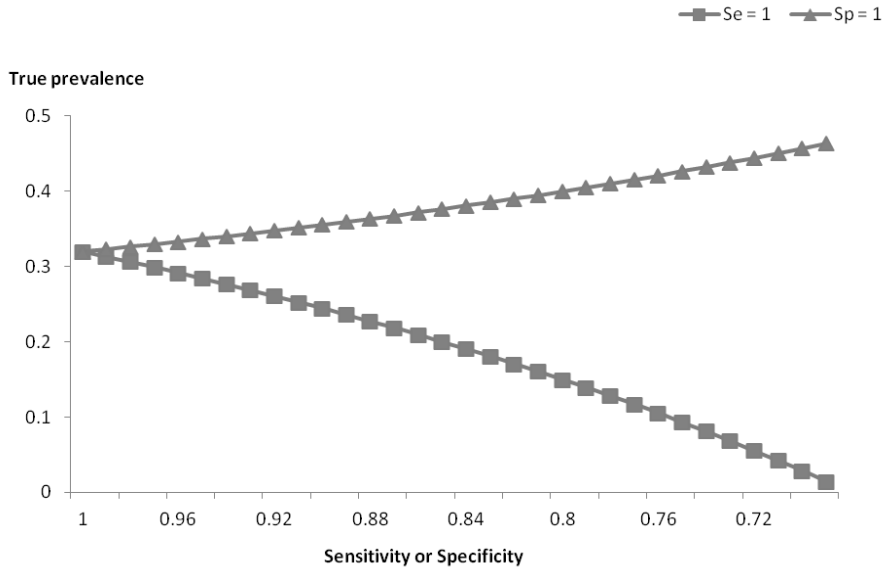

Figure 4.2 Trend of the true prevalence of infection depending on imperfect specificity (*Se* = 1) or imperfect sensitivity (*Sp* = 1) for the observed prevalence of bacteria positive milk samples (p' = 0.32).

If the *Se* is less than unity, then the true prevalence will have been underestimated, whereas if *Sp* is less than perfect then the true prevalence will have been overestimated.

Not only does the true prevalence of infection changes as *Sp* or *Se* change, but the estimated true difference in SCS between healthy and infected animals also changes, as shown in Figure 4.3. Less than perfect *Se* has little impact on the true difference between healthy and infected animals, whereas if *Sp* is less than perfect then the true difference between healthy and infected animals will have been underestimated. Moreover, once *Sp* drops below ~ 0.8 the estimated differences between the two populations becomes improbably large.

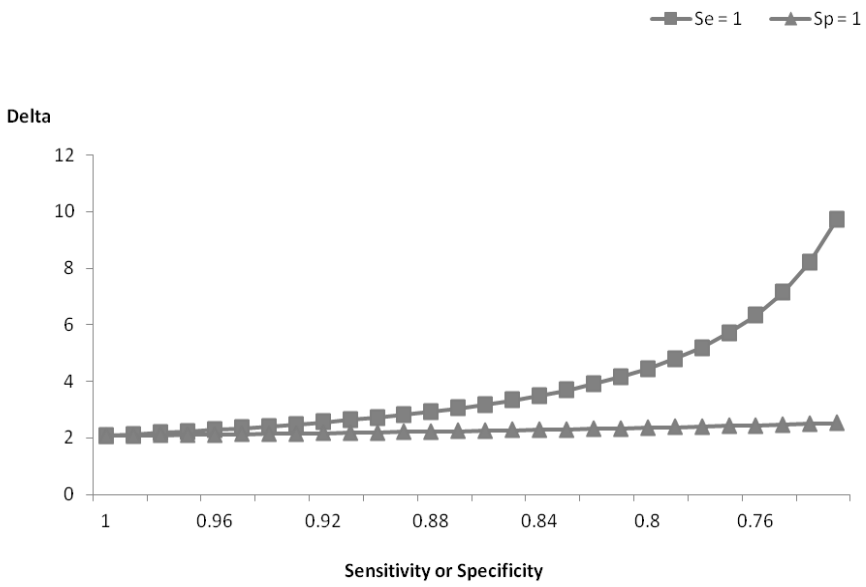

Figure 4.3 Trend of the true difference (Delta) between SCS in healthy and infected populations depending on imperfect specificity (*Se* = 1) or imperfect sensitivity (*Sp* = 1).

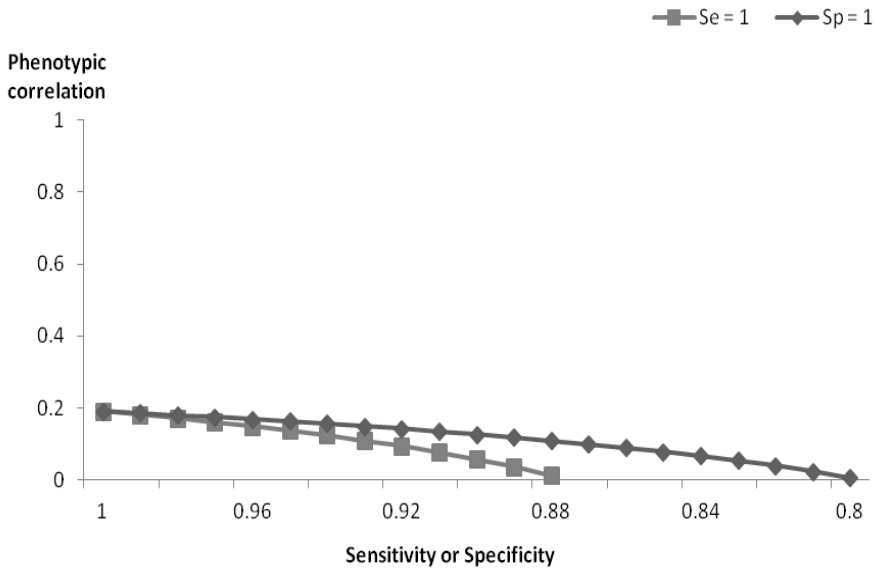

Figure 4.4 Trend of the true phenotypic correlation between SCS in healthy and infected populations depending on imperfect specificity (*Se* = 1) or imperfect sensitivity (*Sp* = 1).

Phenotypic and genetic correlations between SCS in infected and healthy populations also change as *Sp* or *Se* change, as shown in Figures 4.4 and 4.5.

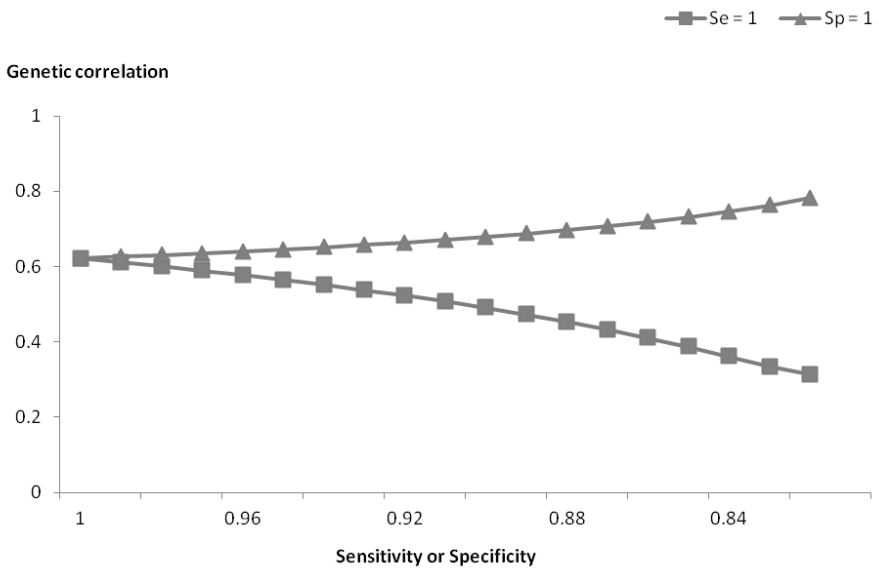

Figure 4.5 Trend of the true genetic correlation between SCS in healthy and infected populations depending on imperfect specificity (*Se* = 1) or imperfect sensitivity (*Sp* = 1).

If both *Se* and *Sp* are less than unity, the true phenotypic correlation will have been slightly underestimated. However, imperfect *Sp* has a larger effect, as the true phenotypic correlation drops more rapidly.

A different trend is reported for the true genetic correlation (Figure 4.5), which will have been underestimated, if *Sp* is less than unity; whereas if *Se* is less than perfect then true genetic correlation will have been overestimated. Although *Sp* and *Se* are unknown in these data, the improbable expected results when either or both values are low suggest that both parameters are likely to be somewhat higher than 0.8.

4.4 Discussion

This paper demonstrates that SCC, and therefore SCS, of apparently uninfected and infected animals are most likely two different traits with different heritabilities. We have shown that bacteria negative SCS has a slightly higher heritability than the infection status (i.e., likely mastitis) and that bacteria negative SCS (i.e., from apparently uninfected animals) is positively genetically correlated with both

bacteria positive SCS (i.e., from infected animals) and infection status. Finally, we have explored the implications of less than perfect *Se* and *Sp* on our estimates. Possibly the greatest impact of less than perfect diagnosis is on the heritability of liability to mastitis, which is likely to be somewhat underestimated if the diagnostic test is poor. This is likely to decrease potential genetic progress for improved resistance.

Evidence has been published that healthy ewes normally have higher SCC than healthy cows (Maisi et al., 1987; Fthenakis et al., 1991; González-Rodriguez et al., 1995). Bufano et al. (1996) have shown that high SCC (> 1 million/mL) do occur in healthy sheep's milk, especially towards the end of lactation. Therefore, whereas in cattle SCC is widely recognized as indicator of mastitis, results on the efficiency of SCC as an indicator trait are inconsistent in dairy sheep studies. However, Ariznabarreta et al. (2002) and Gonzalo et al. (2002) have demonstrated that for around 70% of mammary pathogens isolated from ewes with subclinical mastitis, their presence in ewe milk is associated with high SCC. Therefore, published evidence exists that mastitis does accompany an increase in SCC in sheep (Leitner et al., 2003). Moreover, Leitner et al. (2008) have suggested that because sheep have only two mammary glands, dilution effects due to the mixing of milk with high SCC from an infected gland, and milk with low SCC from a healthy gland, will be relatively small at the animal level. Besides, in dairy cows, subclinical mastitis, with a frequency ranging from 20–50% (Persson-Waller et al., 1997; Wilson et al., 1997) may be less apparent because the increase in SCC in an infected gland is modest (about 300–500 × 10^3 cells/mL) and the mixing with the milk from uninfected quarters is sufficient in most cases to appreciably lower the effect of SCC at the cow level (Djabri et al., 2002).

The mean SCS for bacteria negative animals was similar to the value of 4.86 reported by Ariznabarreta et al. (2002) and 5.15 reported by Leitner et al. (2003); whereas the mean SCS for infected animals was similar to the value of 6.32 reported by Leitner et al. (2003) in Israeli-Assaf and Awassi sheep. The observed difference between the bacteria positive and negative populations was 2.08, i.e., suggesting a four-fold difference in SCC between typical diseased and healthy individuals. However, if only one half of the udder was infected, then due to the dilution this would equate to an eight-fold difference between healthy and infected halves, assuming independence (i.e., infection in one half, which results in an increase in SCC, does not increase SCC in the other half). If *Se* was in fact less than perfect, this would only have slightly influenced the true difference (delta) between the two populations; whereas if *Sp* was less than perfect (i.e., healthy animals

wrongly classified as being infected) then the difference between the two populations would have been considerably underestimated.

The heritability estimates for overall SCS and SCS in apparently healthy animals were generally in the range reported in the literature for repeatability test-day models i.e., 0.04 to 0.16 (Baro et al., 1994; Hamann et al., 2004; Riggio et al., 2007). Other studies have reported higher heritability estimates for the average SCS during lactation, from 0.11 to 0.18 (Mavrogenis et al., 1998; Barillet et al., 2001; Rupp et al., 2001). However, the heritability for SCS in infected ewes (0.03) was at the low end of published values. It is important to highlight that the similarity between the heritability for bacteria negative SCS and that usually observed for SCS is probably due to the fact that the former refers to a mix of repeatable healthy animals, animals that have recovered from infection, and infected animals with incorrect diagnosis. On the contrary, SCS in infected animals are mostly truly positive samples, and the low heritability actually reflects that most of the variation in these samples is non-genetic. The high environmental variance for the bacteria positive SCS is possibly due to the nature of the pathogens (i.e., hosts may respond differently to infection by a pathogen or another) and the sinusoidal variation of SCC after infection, both of which would increase variation in the dataset.

Estimated repeatabilities were similar for the two sub-datasets. Repeatability estimates within lactations ranged between 0.20 and 0.29, and were in the range reported in the literature for sheep i.e., 0.22 to 0.38 (El-Saied et al., 1998; Serrano et al., 2003; Hamann et al., 2004). However, repeatability estimates across lactations ranged between 0.30 and 0.33, and were higher than the value of 0.13 reported by Serrano et al. (2003) for the Manchega breed.

The estimated genetic correlation between bacteria negative and bacteria positive SCS (0.62) is positive and moderate, but significantly less than unity. Therefore, our results suggest that bacteria negative and bacteria positive SCS may be partially independent traits, possibly with different heritabilities. It might be hypothesized that ewes with high bacteria negative SCS also have a higher reaction, in terms of increase in SCS, in response to an infection. It has to be taken into account that the genetic correlation might partially reflect the fact that the dataset of bacteria negative SCS animals also includes previously infected animals. However, a somewhat different interpretation is possible. The bacteria positive SCS actually consists of the bacteria negative SCS (i.e., the SCS ewes would have had in the absence of infection) along with the true response to infection. Therefore, it is likely that the positive genetic correlation is picking up the baseline that is contributing to both measures, with the true response (i.e., the extra) SCS possibly being uncorrelated. The sum of the two results in a trait that is genetically

correlated with bacteria negative SCS, but has a low phenotypic correlation (0.19). The exploration of sensitivity and specificity suggests that imperfect diagnosis of the infection has only minor impacts on the correlation, with the impacts becoming large only when the diagnostic tests are very poor.

Very few data on intramammary infection assessed by bacteriological analyses are found in the literature, and published studies refer more directly and exhaustively to udder health status. In cattle, heritabilities for intramammary infection varied from 0.02 to 0.04 as reported by Weller et al. (1992), and were somewhat higher (0.10 to 0.20) in Detilleux et al. (1994) and Wanner et al. (1998). Our value of 0.09 falls into the mid range of published values. However, an important result we found was that with imperfect *Se* and, particularly, *Sp,* the heritability of liability is likely to be substantially underestimated. In other words, there may truly be more genetic variation for liability to mastitis than the field data suggest. No estimates, however, are reported for the genetic correlation between bacteria negative SCS and the infection status. Our results, perhaps surprisingly, suggest a positive genetic correlation between bacteria negative SCS and liability, suggesting that animals with higher bacteria negative SCS are more liable to have mastitis. This is a result that requires independent validation but it does suggest that the approach of selecting animals for decreased SCS, even in the absence of knowledge about infection status, is correct and will help to reduce the prevalence of mastitis.

The choice of diagnosis criteria is important, as it affects the probability that healthy animals are truly diagnosed as healthy and that infected animals are classified as such. Therefore, as our results have shown, biases may be quite large when diagnostic criteria are not sensitive or specific enough. Our results show that the imperfect diagnosis of infection has an impact on estimated genetic parameters, particularly the heritability of liability, and some of the inferences drawn from the data. Bacteriological examination is often considered to be the 'golden standard' for routine detection and identification of major mastitis pathogens, and is usually assumed to be perfect, i.e., $Sp = Se = 1$. However, even good quality bacteriological or clinical mastitis data will most likely have true *Se* and *Sp* values somewhat less than one. Some cases will be missed, others may be mis-diagnosed. Hence, the answers we get may not be quite what we think they are, and we may well be underestimating the impacts of mastitis and the potential for selecting animals for enhanced resistance.

4.5 Conclusions

Our results suggest that bacteria negative and bacteria positive SCS may be partially independent traits, confirming that SCC from healthy and infected animals should be analyzed separately. Moreover, a positive genetic correlation between bacteria negative SCS and liability to mastitis was found, suggesting that the approach of selecting animals for decreased SCS will help to reduce the prevalence of mastitis. However, our results show that the imperfect diagnosis of infection has an impact on estimated genetic parameters. Hence, the impacts of mastitis and the potential for selecting animals for enhanced resistance may well be underestimated.

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Additional File 1

Effect of imperfect sensitivity and specificity on means and variances of continuous traits

Proportions of animals classified as healthy or diseased, as a function of specificity (*Sp*) or sensitivity (*Se*), where *p* and *p'* are the true and observed prevalence, are:

		Test Classification		
		H[2]	D[3]	Sum
Dis.[1]	H[2]	$(1-p)Sp$	$(1-p)(1-Sp)$	$1-p$
	D[3]	$p(1-Se)$	pSe	p
	Sum	$1-p' = Sp + p(1-Sp-Se)$	$p' = (1-Sp) + p(Sp+Se-1)$	

[1]Dis. = Disease; [2]H = Healthy; [3]D = Diseased

1) The true prevalence is $p = \frac{p' - (1 - Sp)}{Se + Sp - 1}$

2) Let H and H' signify true and observed healthy animals, and D and D' signify true and observed diseased animals. The means for the observed healthy and diseased populations can be considered as weighted averages of the true population means:

$\overline{H}' = X_1\mu_H + X_2\mu_D$ and $\overline{D}' = X_3\mu_H + X_4\mu_D$

where:

$X_1 = \frac{(1-p)Sp}{1-p'}$, $X_2 = \frac{p(1-Se)}{1-p'}$, $X_3 = \frac{(1-p)(1-Sp)}{p'}$, and $X_4 = \frac{pSe}{p'}$

Therefore, the true difference $(\mu_D - \mu_H = \Delta)$ between diseased and healthy animals, after noting that $X_1 = 1 - X_2$ and $X_3 = 1 - X_4$, and after simplification, is given by:

$\Delta = (\mu_{D'} - \mu_{H'})\{(Sp + Se - 1)p(1-p)/[p'(1-p')]\}^{-1}$

Solving for *p*, this formula may be rewritten:

$\Delta = (\mu_{D'} - \mu_{H'})p'(1-p')(Sp + Se - 1)/[(p' + Sp - 1)(Se - p')]$

3) The variance of H' and D' are variances of distributions comprising different proportions healthy and diseased animals. In general, a mixture of 2 distributions Y and Z, in proportions *p* and *(1-p)* will have a variance:

$p\sigma_y^2 + (1-p)\sigma_z^2 + p(1-p)(\mu_y - \mu_z)^2$.

Therefore, the true variances for healthy and diseased animals can be deduced from the following equations:

$V(H') = X_1\sigma_H^2 + X_2\sigma_D^2 + X_1X_2\Delta^2$ and

$V(D') = X_3\sigma_H^2 + X_4\sigma_D^2 + X_3X_4\Delta^2$

4) For the covariance of H' and D', comparing the same individual, we have 4 possibilities:

Classification		
Healthy	Diseased	Frequency
True healthy	True healthy	X_1X_3
True healthy	True diseased	X_1X_4
True diseased	True healthy	X_2X_3
True diseased	True diseased	X_2X_4

The covariance is therefore:

$$Cov(H', D') = X_1 X_3 \sigma_H^2 + X_2 X_4 \sigma_D^2 + (X_1 X_4 + X_2 X_3)\sigma_{H,D}$$

From this equation it is possible to deduce the true covariance between healthy and diseased animals, and the implied true correlation can be constructed from the estimated true variances and true covariance terms.

5

Receiver-operating characteristic curves for somatic cell scores and California mastitis test in Valle del Belice dairy sheep

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Abstract

The aim of this research was to apply the Receiver-Operating Characteristic (ROC) curve methodology to evaluate the diagnostic effectiveness of SCC and CMT and to propose and evaluate threshold values of SCC and CMT in the Valle del Belice dairy sheep. The data consisted of 1,357 milk samples from 684 Valle del Belice dairy sheep collected in four flocks. In 83.7% and 97.4% of uninfected samples, SCC were less than 500 and 1,000 x 10^3 cells/mL, respectively. Considering the whole sample, the prevalence of infection was 36.4%, of which 87.7% represented by minor pathogens and 12.3% by major pathogens. The semi-parametric and non-parametric estimates of the area under curve (AUC) were similar with a tendency for non-parametric estimates of being higher than semi-parametric estimates, except for CMT diagnostic test, which showed a slightly lower value for semi-parametric estimate. The estimated AUC was greater for glands infected with major pathogens than for glands infected with minor pathogens (0.88 *vs.* 0.73), whereas the AUC considering all pathogens was similar to the one with minor pathogens (0.75). The four diagnostic tests (CMT, SCS, SCS_MIN, and SCS_MAJ) for each threshold value, allowed correctly classifying, 69.0, 73.5, 72.6 and 91% of infected udders, respectively. The analysis of the AUC's provided useful information which enables to optimize the use of a test through targeted selection of threshold values for each diagnostic strategy considered.

Key words: intramammary infection, receiver operating characteristic curve, sheep

5.1 Introduction

Intramammary infections (IMI) are the main cause of mastitis in both dairy ewes and cows. Mastitis is an inflammation of the udders and leads to economic losses, mainly consisting in discarded milk, reduced milk production and quality, and increased health costs (i.e., Miller et al., 1993; Allore and Erb, 1998; Leitner et al., 2004). Direct bacteriological assay is considered to be the most reliable method of diagnosis of mastitis (González-Rodríguez and Cármenes, 1996), because it provides precise and exhaustive information on infected quarters and pathogens involved. The bacteriological examination is indeed often considered to be the 'golden standard' for routine detection and identification of mastitis pathogens, and, therefore, is usually assumed to provide a perfect diagnosis. However, it is rarely used for genetic purposes, because it is difficult to implement at a large scale and it has limitations because of the requirement for laboratory support, the time delays for culture to occur, and the costs associated with bacteriology (McDougall et al., 2001). Simple indirect methods have been widely applied, based on the evaluation of the degree of inflammation or of internal mammary lesions (De la Cruz et al., 1994) and their accuracy is usually established by bacteriological analysis as a reference method (i.e., golden standard). Among these methods, the most developed ones are milk somatic cell count (SCC) and the California Mastitis Test (CMT).

Mastitis indeed causes an increase in SCC in both small ruminants (Zeng et al., 1997; Leitner et al., 2004) and cattle (Heringstad et al., 2006). For this reason, SCC and therefore somatic cell score (SCS) have been widely recognized as indicator of mastitis and as selection criterion for improved mastitis resistance (Colleau and Le Bihan-Duval, 1995). Since SCC is measured on continuous scale (i.e., quantitative reading), a value on the original scale has to be selected as decision threshold (cut-off value) to define a positive or negative test-outcome. Whereas in cattle SCC values between 250 and 300 $x10^3$ cells/mL are recommended as most satisfactory discrimination thresholds between healthy and infected udders, in sheep there is no universally accepted threshold.

Under field conditions, the only tool currently used for SCC determination is CMT, because CMT scores are directly related to average SCC. However, CMT, which is based on scoring the degree of gel formation in a milk and bromocresol reagent mixture, is a subjective screening test. This method is easy and inexpensive enough for dairymen to afford and, although there is the implied problem of being a subjective test, it is considered an indicator of subclinical mastitis in cattle. In the classification reported by Kivaria et al. (2007), when the CMT score is 0 (i.e.,

negative or trace), the equivalent SCC is between 0 and 200 $x10^3$ cells/mL; when the score is 1, SCC is between 150 and 500 $x10^3$ cells/mL; when the score is 2, SCC is between 400 and 1,500 $x10^3$ cells/mL; and when the score is 3, SCC is between 800 and 5,000 $x10^3$ cells/mL. However, other relationships have been proposed by other authors (e.g., McDougall et al., 2001).

In sheep, some authors have reported a positive association between the CMT score, SCC, and the probability of bacterial infection (Maisi et al., 1987; Contreras et al., 1996; Gonzalez-Rodriguez and Carmenes, 1996). However, the usefulness of CMT as indicator of subclinical mastitis is still doubtful, as healthy sheep normally have higher SCC than cows (Maisi et al., 1987; Ftenakis et al., 1991; Gonzalez-Rodriguez et al., 1995).

The choice of a threshold value to distinguish healthy from infected animals is likely to be important in the identification of effective strategies for the selection for increased mastitis resistance. Nevertheless, SCC and CMT diagnostic effectiveness (i.e., SCC and CMT ability to detect whether or not IMI occur) may be assessed to a degree without having to commit to a single threshold with the use of average indices based on Receiver-Operating Characteristic (ROC) curves. The ROC methodology was developed in the early 1950s for the analysis of signal detection in technical sciences and was first used in medicine in the late 1960s for the assessment of imaging devices (Greiner et al., 2000). At present, ROC curves are widely accepted as the standard method for describing and comparing the accuracy of radiologic imaging (Obuchowski et al., 2004) and other medical diagnostic tests (Metz, 1986; Zhou et al., 2002; Pepe, 2004; Wagner et al., 2007) when the localization of a disease is not essential to its evaluation. An ROC curve is a plot of a test's true-positive fraction (TPF) *vs.* false-positive fraction (FPF) for each possible test result value, indicating all tradeoffs between sensitivity (*Se*) and specificity (*Sp*, 1 - FPF) that are available (or have been observed if the ROC curve is empirical as is usually the case). Bamber (1975) and Hanley and McNeil (1982) recognized that the Area Under Curve (AUC) is equivalent to the probability that a randomly drawn individual from the positive reference sample has greater test value than a randomly drawn individual from the negative reference sample (when positivity is assumed for larger values, as in this manuscript, meaning that the test is operated assuming that greater values are associated with the presence of the disease). The AUC from different tests can be compared, with the highest AUC values representing the test or tests having the highest average *Se* over the complete *Sp* range of the test observed in the experiment (De Long et al., 1988; Pepe, 2004). The aim of this study was to apply the ROC curve methodology to evaluate the

diagnostic effectiveness of SCC and CMT and to propose and evaluate threshold values of SCC and CMT in the Valle del Belice dairy sheep.

5.2 Material and methods

All procedures involving animals were performed according to the principles and specific guidelines on animal care and welfare as required by Italian law.

A total of 1,357 milk samples from 684 Valle del Belice dairy sheep belonging to four flocks were collected and analyzed for this study. Although there were repeated measures in the data, all test days were considered as independent from each other because they were relatively infrequent and displayed no pattern and, therefore, we could not reasonably model this effect. We also believed it was reasonable to assume that if a ewe was measured at two different points in time; those two points would have been most of the time essentially independent. Moreover, we were not interested in a time dependent relationship, i.e., relation between two adjacent test days. This should make our statistical testing only slightly anti-conservative. Milk samples were collected during morning milking for SCC determination. At the same time, milk samples were also collected from the two udder halves in sterile containers for bacteriological analyses and kept under 4 °C before processing. Udders were washed and dried before milking as is standard procedure. The bacteriological colonies observed were mainly: *Staphylococcus aureus*, coagulase negative staphylococci, *Staphylococcus intermedius*, and other staphylococci; *Streptococcus uberis*, *Streptococcus agalactiae*, and other streptococci; and *Pasteurella* spp., and were divided in two groups (major and minor) according to their pathogenicity.

SCC were determined with a Fossomatic 5000 (Foss Electric Hillerød, Denmark) counter using the chilled milk samples. Three different SCS traits were considered: SCS for the whole sample (i.e., considering uninfected and infected glands), SCS for minor pathogens (SCS_MIN - i.e., considering uninfected and infected by minor pathogens glands), and SCS for major pathogens (SCS_MAJ - i.e., considering uninfected and infected by major pathogens glands).

The CMT test was carried out according to the manufacturer's instructions, and the scores divided in five categories, in accordance to McDougall et al. (2001): 0 (absence of any variation in viscosity), T (traces: slight slime with disappear), 1 (slime without gel), 2 (appreciate gel), and 3 (gel adhered to the bottom of the cup). Therefore, the values of this test are ordinal categorical: larger values imply higher probability for the presence of the condition, but the intervals themselves have no meaning, i.e., "slime without gel" plus "appreciate gel" does not

correspond to “gel adhered to the bottom of the cup” (Agresti, 2002). However for the features described above, the CMT test is a very subjective diagnostic test, above all for the first three classes 0, T and 1.

Both the bacteriological and the CMT score analyses were performed at the Istituto Zooprofilattico Sperimentale per la Sicilia “A. Mirri”, which is accredited by Sinal (National accreditation system laboratories, now ACCREDIA), on the basis of laboratory technical proficiency in conducting specific tests and on evaluation of the quality system of the laboratory.

SCC were log-transformed to SCS, where SCS = $\log_{10}$(SCC/1000). For this variable a normality-probability plot and a Kolmogorov D statistics were performed (Proc UNIVARIATE, SAS® 9.1.3, 2006) to assess its normality and to define the association degree between the variables test. Spearman's correlation coefficients were also calculated.

Two different approaches were used to fit ROC curves to both continuously-distributed (SCS) and ordinal categorical (CMT) data: a semi-parametric binormal approach (Metz et al., 1998) and a non parametric approach (Bamber, 1975; Hanley and McNeil, 1982). In passing, purely parametric approaches are rarely useful in human or animal medical research because decision variables seldom follow well enough a specific mathematical distribution and it is rarely possible to make a reasonable *a priori* estimate of a transformation capable to produce data with the correct statistical properties. However, it is not infrequent that some, unknown, transformations of those variables will produce the desired outcome. Therefore, in this study it was assumed that SCS data can be mapped to a latent decision-variable distributions of specified form (the method by Metz et al. does not require to pre-specify the mapping, but only that it is monotonic, and it has been used and tested in a large number of experiments), in order to be able to fit a smooth ROC to SCS and CMT outcome data. In this case, the most widely used assumption is the normally distributed values for the two groups of responses (healthy and infected), i.e., the ROC curve plots as a straight line on “*normal deviate*” axes, where the normal deviate corresponding to TPF (*Se*) is plotted *vs.* the normal deviate corresponding to FPF (1-*Sp*).

The binormal assumption has been empirically found to provide satisfactory ROC curves fitting to data in a very broad variety of situations (Swets et al., 1986; Hajian-Tilaki et al., 1997; Metz et al., 1998), with some notable exceptions when the model produces strange looking hooked curves. However, these failures of the model are usually easily spotted and other models can be used to in such situations (Pesce et al., 2010). If an ROC curve is found empirically to plot as a straight line on normal deviate axes, the vertical and horizontal coordinates of each point on that

line are related by: $TPF = \Phi\left(a + b\Phi^{-1}(FPF)\right)$ where Φ is the standard normal cumulative distribution function, *a* is the intercept, and *b* the slope of the straight line plot on normal deviate. The area under binormal ROC curve, denoted by A_z is related to the curve parameters *a* and *b* by $A_Z = \Phi\left(\frac{a}{\sqrt{1+b^2}}\right)$. The assumption that an ROC has binormal form is substantially less strict than the assumption that the explicit decision-variable distributions are normal. Moreover, Metz et al. (1998) have shown that maximum likelihood (ML) estimation of an ROC curve for ordinal categorical data can be applied to continuously-distributed data after replacing the latter with their truth-state runs (i.e., contiguous sequences of values which can be positive or negative, but not both – unless they have the same value; for details see Metz et al., 1998). The software ROCKIT 1.1.β2 or roc.jar (both available without charge from http://metz-roc.uchicago.edu/MetzROC/software) are available to perform this type of analysis.

Using the non-parametric approach, the empirical *Se* and *Sp* were derived by dichotomizing the observed (empirical) values into positive or negative test-results for each observed cut point *z* (here *z* is used to indicate the observed variable – i.e., SCS or CMT). Now, as *z* varies over the observed values of the variables, the empirical ROC curve is defined as the discrete set of $Se_{(z)}$ and [1-$Sp_{(z)}$] values joined by straight lines (Pepe, 2004). Clearly, when *z* is larger than the maximum value observed, the curve passes through point (0,0), whereas it monotonically increases to the point (1,1), as *z* decreases to the smallest possible value. To be informative, the curve should be above 45° line at least for some of the values, where $Se_{(z)}$ is equal to 1-$Sp_{(z)}$ (Pesce et al., 2010). In the non-parametric approach, the AUC can be estimated by the trapezoid defined by the empirical set of $Se_{(z)}$ and [1-$Sp_{(z)}$] values. However, since $AUC = \theta = \text{Prob}\left(x_D > x_H\right) + 0.5\,\text{Prob}\left(x_D = x_H\right)$, with x_D being the samples from the infected population distribution and x_H the samples from the healthy population distribution, its value is related to the *U* statistic for the two-sample Mann-Whitney/Wilcoxon rank-sum test (Bamber, 1975; Hanley and McNeil, 1982). Therefore, in this case the AUC was estimated as: $AUC = \frac{n_H n_D - U}{n_H n_D}$, where n_D and n_H are the sample size of infected and healthy individuals, respectively; $U = R - \frac{1}{2} n_H (n_H + 1)$; and *R* is the rank sum of negative sample. Under the null hypothesis of a non-informative test, the expected value for the rank sum is $E(R) = \frac{1}{2} n_H (n+1)$, where n is the total sample size (n_D+n_H), and therefore,

$U = \frac{1}{2}(n_H n_D)$ and $\theta = 0.5$. The null hypothesis was assessed using the test statistics $Z = \frac{(R - E(R))}{\sqrt{\text{var}(R)}}$. The variance of R was estimated as $Var(R) = \frac{n_H n_D s^2}{n}$, where s^2 is the sample variance of the combined ranks for both groups. After estimating full AUC's and their variances, we evaluated whether the different tests had equal diagnostic capability. If the two tests were used on the same sample (i.e., CMT and SCS for the whole sample), the null hypotheses H_0: $\theta_i = \theta_{i+1}$ for the AUC's were tested with two-sample *Z-test* $Z = \frac{\theta_i - \theta_{i+1}}{\sigma_{(\theta_i - \theta_{i+1})}}$ in which $\sigma_{(\theta_i - \theta_{i+1})} = \sqrt{\sigma^2_{\theta_i} + \sigma^2_{\theta_i+1} - 2rSE\,\theta_i SE\,\theta_{i+1}}$ where *r* is the correlation coefficient between the two samples, whereas for unpaired tests, the formula was just $\sigma_{(\theta_i - \theta_{i+1})} = \sqrt{\sigma^2_{\theta_i} + \sigma^2_{\theta_i+1}}$, i.e., r = 0. The correlation coefficients of pairs of areas and standard error of AUC were derived from estimated covariance matrix obtained using the theory on generalized U-statistics (De Long et al., 1988) and using the roc.jar software (http://metz-roc.uchicago.edu/MetzROC/software).

Finally the ROC curve was used to select threshold values that would yield the optimal mix of FPF and FNF (False Positive and False Negative Fraction, respectively), given a specific diagnostic test, IMI prevalence (P), and costs assigned to false positive (C_{FP}), false negative (C_{FN}), true positive (C_{TP}), and true negative (C_{TN}) results. The average cost ($\overline{C}$) of a diagnostic test is given by:

$$\overline{C} = (TPF * C_{TP} * P) + [FPF * C_{FP} * (1 - P)] + [TNF * C_{TN}(1 - P)] + (FNF * C_{FN} * P),$$

where TPF, FPF, TNF, and FNF are the true positive, false positive, true negative, and false negative fractions, respectively; C_{TP}, C_{FP}, C_{TN}, and C_{FN} are the corresponding costs; and P is the IMI prevalence. By computing the first derivative of the previous expression as a function of the cutoff value and setting it equal to zero, one can find the optimal operating point (OOP). It is straightforward to show that the slope of curve for the optimal operating point, S, has to satisfy $S = \frac{1 - P}{P} * \frac{C_{FP} - C_{TN}}{C_{FN} - C_{TP}}$.

5.3 Results

Descriptive statistics for SCS according to the bacteriological status are shown in Table 5.1. Mean for SCS of 863 uninfected samples was equal to 2.13 and lower

than the value considering all 494 infected (i.e., minor plus major pathogens) ones (2.81, results not shown).

Table 5.1 Descriptive statistics of milk samples according to bacteriological status.

	All samples	Culture Negative	Minor Pathogens	Major Pathogens
N. Samples	1,357	863	433	61
Mean SCS	2.38	2.13	2.73	3.35
Standard Deviation of SCS	0.78	0.65	0.79	0.67

However, given that we had only information at the animal level (i.e., summarizing the whole udder) a dilution effect due to the mixing of milk with high SCC coming from an infected gland and milk with low SCC from a healthy gland has to be taken into account. The difference between uninfected and infected sample SCS, therefore, may have been higher if samples had been collected per udder half. However, we believe that a test that does not require a separation of the milk has more practical interest and this is the reason why it was pursued here. The highest SCS mean was found for glands infected with major pathogens (3.35).

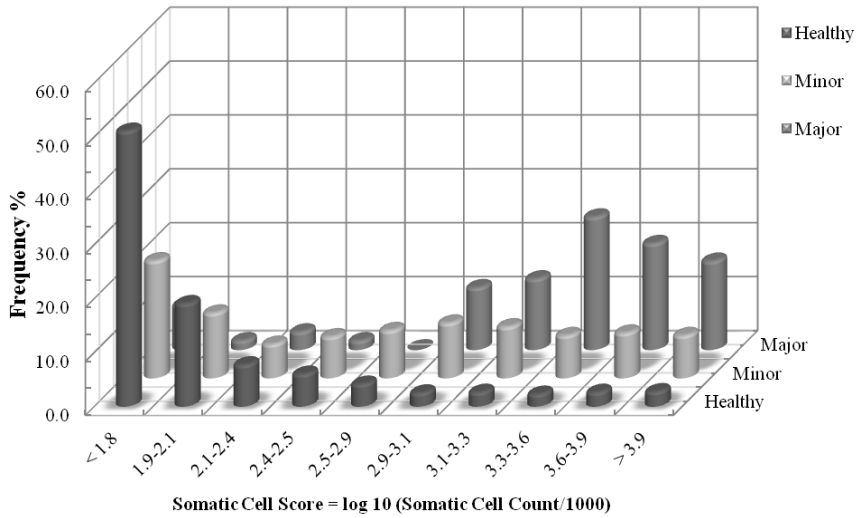

Figure 5.1 Distributions for healthy and infected glands by minor and major pathogens.

In 83.7% and 97.4% of uninfected samples, SCC were less than 500 and 1,000 x10^3 cells/mL, respectively. Considering the whole sample, the prevalence of infection was 36.4%, of which 87.7% represented by minor pathogens and 12.3% by major pathogens. Distributions of SCS for uninfected and infected (both by minor and major pathogens) udders are shown in Figure 5.1.

Using the Kolgomorov D statistic test for goodness-of-fit to the normal distribution, the probability values for the distribution of negative bacteriological test for the other two distributions of positive bacteriological classes were $P<0.01$, indicating that even after the log transformation, neither distribution was normal, which confirms that simple parametric methods are inappropriate for our data.

Table 5.2 reports the arithmetic mean of SCS, ranging from 2.00 to 4.00 in CMT classes, whereas the geometric mean for SCC ranged from 100.13 to 9,955.56 (x 10^3 cells/mL). A moderate and positive as well as significant ($P<0.001$) Spearman correlation existed between SCS and CMT ($r = 0.62$).

Table 5.2 Arithmetic mean and standard deviation (SD) of SCS and geometric mean and SD of SCC (x10^3 cells/mL) for CMT scores.

CMT	N	Arithmetic Mean	SD	Geometric Mean	SD
0	837	2.00	0.45	100.13	2.81
T	149	2.44	0.62	274.91	4.17
1	172	2.66	0.69	456.97	4.92
2	116	3.43	0.52	2681.02	3.34
3	83	4.00	0.35	9955.56	2.23

The ROC curves for the different diagnostic tests, i.e., CMT, SCS for the whole sample, SCS_MIN, and SCS_MAJ are shown in Figure 5.2. As an example, for SCS=2.0, the observed *Sp* was equal to 0.58 (CI = 0.55-0.61) for SCS, SCS_MIN, and SCS_MAJ, whereas *Se* was 0.77 (CI=0.73-0.81), 0.75 (CI=0.71-0.79), and 0.92 (CI=0.82-0.97) for SCS, SCS_MIN, and SCS_MAJ, respectively.

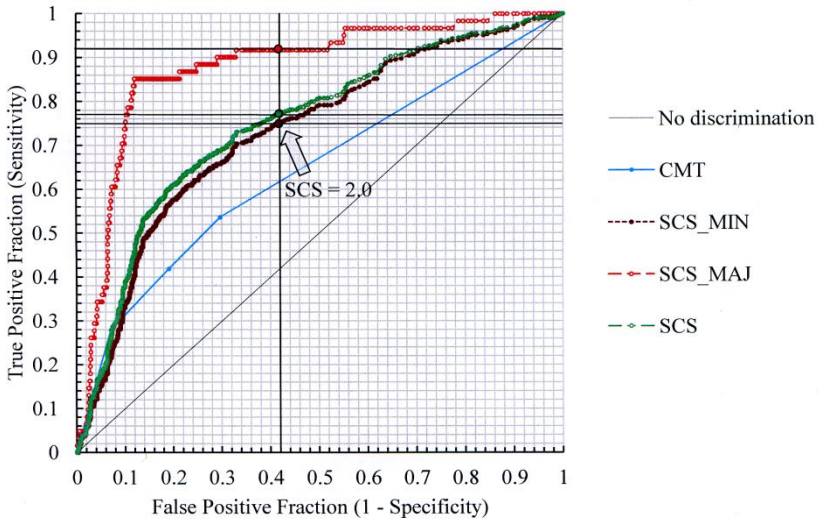

Figure 5.2 ROC curves illustrating the performance of SCS and CMT in identifying infected glands with minor and major pathogens.

The estimated AUC was greater for glands infected with major pathogens (AUC=0.88) than for glands infected with minor pathogens (AUC=0.73), whereas the AUC considering all pathogens (AUC=0.75) was similar to the one with minor pathogens (Table 5.3).

Table 5.3 AUC and standard error (S.E.) estimates for CMT, SCS, SCS_MIN, and SCS_MAJ diagnostic tests.

	CMT		SCS		SCS_MIN		SCS_MAJ	
	AUC	**S.E.**	**AUC**	**S.E.**	**AUC**	**S.E.**	**AUC**	**S.E.**
Wilcoxon	0.64	0.014	0.75	0.014	0.73	0.015	0.88	0.024
$A_{(z)}$	0.66	0.021	0.74	0.014	0.72	0.015	0.87	0.020

The observed curve for the major pathogens actually dominates the curve for the minor ones and CMT (AUC=0.64) for nearly all the range, and overlaps with them only at very low values of FPF. The probabilities of the null hypothesis H_0 that AUC's of CMT and SCS for all three infection classes (all pathogens, minor, and major) were equal to 0.5, were $P<0.0001$ (Table 5.4).

Table 5.4 Value statistic *Z-test* used for testing the null hypothesis for CMT, SCS, SCS_MIN, and SCS_MAJ.

TEST	CMT	SCS	SCS_MIN	SCS_MAJ
Wilcoxon	9.99*	17.76*	15.37*	16.13*
$A_{(z)}$	7.62*	17.14*	14.67*	18.50*

* P<0.0001

This means that the null hypothesis can be rejected, i.e., SCS and CMT are laboratory tests having the ability to better than randomly distinguish between non-infected and infected udders. The non-parametric comparison between the AUC's are reported in Table 5.5.

Table 5.5 Non parametric comparison between the AUC's of different diagnostic tests.

Contrast	Difference	SE	Z	P
CMT *vs.* SCS	-0.107	0.012	-8.87	<0.000
CMT *vs.* SCS_MIN	-0.087	0.013	-6.61	<0.000
CMT *vs.* SCS_MAJ	-0.235	0.026	-8.99	<0.000
SCS *vs.* SCS_MIN	0.020	0.020	0.98	<0.248
SCS *vs.* SCS_MAJ	-0.128	0.027	-4.70	<0.000
SCS_MIN *vs.* SCS_MAJ	-0.148	0.028	-5.33	<0.000

The probability for the hypothesis that SCS_MAJ has greater AUC than SCS_MIN was P<0.0001, as well as those for SCS_MAJ *vs.* CMT and SCS_MIN *vs.* CMT. The same results were obtained from the semi-parametric comparison of the maximum likelihood estimates of the binormal parameters for the estimated ROC curves of SCS and CMT (Table 5.6). The p-values should be small enough to compensate for the slightly anti-conservative nature of a test that ignores that some animals might have been tested more than once. Note that for the subjective test additional variability exists between people performing the test; however, given the large number of tests and observers and the high significance of the test, we expect its effect on our conclusions to be negligible.

Table 5.6 Semi-parametric comparison of Maximum likelihood estimates of the binormal parameters between estimated ROC curve of SCS and CMT.

Contrast	Parameter				χ_1^2 value	P
	a	b	a	b		
CMT *vs*. SCS	0.46	0.77	0.89	0.93	43.94	>.00
CMT *vs.* SCS_MIN			0.88	0.94	36.85	>.00
CMT *vs.* SCS_MAJ			2.07	0.94	114.08	>.00
SCS *vs.* SCS_MIN	0.91	0.97	0.91	0.98	1.22	>0.54
SCS *vs.* SCS_MAJ			1.54	1.36	187.29	>.00
SCS_MIN *vs.* SCS_MAJ	1.27	1.21	2.68	1.49	453.60	>.00

Despite its high economic impact, there is very little information about mastitis incidence and economic consequences in sheep breeds. Actually, costing of farm animal diseases is not simple and the calculations may be misleading and lead to suboptimal decision-making, i.e., economic loss. Few of the estimates of the costs of specific diseases follow good practice, usually owing to a lack of appropriate data (Bennett, 2003).

To determine the Optimal Operative Point (OOP) on ROC curve, the costs associated to true and false positive (C_{TP} and C_{FP}) and negative (C_{TN} and C_{FN}) results were estimated (Table 5.7).

Table 5.7 Costs associated to four different test diagnostic results.

	C_{TP} (€)	C_{FP} (€)	C_{TN} (€)	C_{FN} (€)
SCS	50.00	75.00	12.50	99.75
SCS_MIN	50.00	75.00	12.50	99.75
SCS_MAJ	50.00	75.00	12.50	200.00

Costs for the four different diagnostic test results were estimated considering different costs for pathogen class (minor and major). The base cost of mastitis was split into veterinary treatments, discarded milk, and farmer's time. Moreover, a cost for fatality was considered for major pathogens. Concerning the treatments, it was assumed a cost of €20 for the vet plus a cost of €20 per animal for medications. Because of the treatment, milk has to be discarded during the treatment days and waiting time. We assumed that milk had to be discarded for 6 d

(Huijps et al., 2008) with a cost of €7. The cost for farmer's time was assumed to be €3.

Table 5.8 SCS thresholds yielding highest percentages of correctly classified results for each diagnostic test considered.

	P	SCS	SCC	OOP		LR			PPV	NPV
				Se	*Sp*	*+*	*p*	*-*		
CMT=2	0.36	≥3.00	≥ 2.4x10^6	0.28	0.93	3.95	2.19	0.78	0.69	0.69
SCS	0.36	2.81	645,000	0.53	0.86	3.87	2.19	0.54	0.69	0.76
SCS_MIN	0.33	2.81	645,000	0.48	0.86	3.54	2.50	0.60	0.64	0.77
SCS_MAJ	0.07	3.33	2,137,962	0.61	0.93	8.30	5.89	0.42	0.37	0.97

Decision thresholds are shown in Table 5.8 for the observed IMI prevalence, considering costs reported in Table 5.7. Table 5.8 also shows the likelihood ratios (LR) and positive and negative predicted values (PPV and NPV, respectively). The decision threshold was determined for each diagnostic test as the value that yields the optimal mix of FPF and FNF, minimizing the cost function S, defined above. The OOP for CMT presented the score ≥2, which corresponds to SCS≥3.00 and to SCC≥2.4 x10^6. The threshold value for SCS, considering P=0.36, had *Se*=0.52 and *1-Sp*=0.13. In this case, the OOP corresponds to the decision threshold of 2.81 SCS. For SCS_MIN the cost function *S* is minimized at the point with *Se*=0.48 and *1-Sp*=0.14, which corresponds to the decision thresholds of 2.81 SCS; whereas, the decision threshold of SCS_MAJ is 3.33, being the point on the corresponding ROC plot with *Se*=0.61 and *1-Sp*=0.03.

The PPV (and NPV) values at decision thresholds were 0.69 (0.69), 0.69 (0.76), 0.64 (0.77), and 0.37 (0.97) for CMT, SCS, SCS_MIN, and SCS_MAJ, respectively. Three LR are reported in Table 5.8. The LR+ and (LR-) were 3.95 (0.78), 3.87 (0.54), 3.54 (0.60), and 8.30 (0.52) for CMT, SCS, SCS_MIN, and SCS_MAJ, respectively. For a specific value *x* of the test, the LR is the slope of the line tangent to the ROC curve at the corresponding point on the curve. These values for the three considered diagnostic tests were 2.19, 2.19, 2.50 and 5.89.

5.4 Discussion

The mean SCS for uninfected udders was lower than the values of 4.86 reported by Ariznabarreta et al. (2002) and 5.15 reported by Leitner et al. (2003); whereas the

mean SCS for the infected ones was lower than the value of 6.32 reported by Leitner et al. (2003).

The ROC analysis indicated that CMT and SCS perform significantly better than by chance as diagnostic tests for IMI detection. As shown with both the Kolmogorov D statistics and Figure 5.1, the assumptions of the parametric binormal model were violated for all diagnostic tests. For this reason we used the semi-parametric and non-parametric approaches to estimate AUC's. Distributions of the SCS of infected and uninfected glands are different but overlapping (Figure 5.1), suggesting that SCS should discriminate better than by chance. This was verified with semi-parametric and non-parametric methods for all diagnostic tests with AUC estimates greater than 0.5. The semi-parametric and non-parametric estimates of the AUC's reported in Table 5.3 were similar with a tendency for non-parametric estimates of being higher than semi-parametric estimates, as already reported by Detilleux et al. (1999), except for CMT diagnostic test, which showed a slightly lower value for semi-parametric estimate.

Applying any multiple comparison method, i.e. Holm's method, (Holm, 1979), we obtained that all differences were simultaneously statistically significant, i.e. we are confident at 95% that they are ranked as SCS_MAJ>SCS_MIN=SCS>CMT. These results are confirmed by the comparison between the AUC's of the different diagnostic tests, obtained by means non-parametric and semi-parametric methods. However, whereas it is highly probable that the SCS_MAJ test is superior to CMT for all the possible values (N, T, 1, 2, 3 *vs.* comparable SCS), it seems likely that CMT might be superior to SCS and SCS_MIN if used at score 2.

The costs associated to the four different diagnostic test results were estimated, considering some simplifications. We assumed that a ewe gets only one clinical or subclinical mastitis case, so repeated cases were not taken into account, whereas this can have an effect on the milk production losses (Bar et al., 2007). Farmers' labour was another cost difficult to assess, because most of the Sicilian flocks are family-run and because the work associated with mastitis is regarded as annoying (Kuiper et al., 2005). In addition, we assumed no extra culling of ewes for subclinical mastitis.

Moreover, in the analysis of the costs, a higher cost value was attributed to the false negative results. This occurred, as a false negative result determines more economic losses than a false positive result. Nevertheless, false positive results also cause unnecessary treatments on animals and disposal of milk (Radostits et al., 1999). For these reasons, we used dynamical approach and multiple thresholds (Bergonier et al., 2003a). The priority to define threshold values was given to major cost determined from false negative results for the IMI caused by minor and major

pathogens and to reduce the economic impact of unnecessary treatments and disposal of milk of the ewes' false positives.

So far, CMT and/or SCS diagnostic capability was not assessed choosing any cut-off point. In a given clinical or subclinical situation and for observed IMI prevalence, efficacious CMT or SCS threshold should be chosen by weighing consequences of misclassification. Highest percentages of correctly classified results are determined by the point on ROC curve (OOP), where a straight line with a slope equal to the cost function *S* is tangential to the ROC curve. The estimated OOP on the ROC curve with defined costs associated to the four different results of a diagnostic test moves towards higher *Se* and lower *Sp* when C_{FN} increases (as in SCS_MAJ) respect to $C_{FP,}$ or when IMI prevalence increases as in CMT, SCS, and SCS_MIN. The *Se* and *Sp* values for cut-off point for CMT diagnostic test were slightly higher than those reported by McDougall et al. (2001) in goats. For CMT score equal to 2, the SCS value was higher than those for SCS for the whole sample and SCS_MIN (Table 5.8). Practically, this means that CMT cannot discriminate between healthy and infected udders. This is confirmed by the higher value of *Sp* and lower value of *Se*.

The same threshold value (2.81) was found for SCS and SCS_MIN threshold, suggesting that this threshold has the same power to discriminate the healthy udders from those infected for the two diagnostic tests. The slightly higher *Se* of the SCS is due to the slightly higher prevalence of SCS. This SCS value corresponds to 645 x 10^3 cells/mL SCC. In ewes with fluoro-opto-electronic method, the single thresholds proposed range from 200 x 10^3 to 1.5 x 10^6 cells/mL; nevertheless, the majority of them are below 500 x 10^3 cells/mL (Bergonier et al., 2003a). Some authors suggested using two threshold to distinguish "healthy" from "infected" udders (140 x 10^3 and 340 x 10^3, respectively) (Romeo et al., 1998) and "minor" from "major" pathogen-causing IMI (244 x 10^3 and 10^6 cells/mL) (Suarez et al., 2002). Observed *Se* for all *Sp* were rather low because in the minimization of the cost function *S*, higher weight was given to the false negative costs rather than to false positive costs, i.e., major economic losses are due to the cost of false negative results. Moreover, the economic losses for subclinical mastitis were estimated to be higher than those for clinical mastitis. However, concerning the economic losses for clinical mastitis, it should be taken into account the greater risk of fatality, which is therefore included in the false negative costs for this category.

In our study, prevalence ranged from 0.07 (considering only major pathogens) to 0.36 (considering the whole sample). These values are comparable with those reported by Bergonier et al. (2003b). In the current study, when prevalence is low as in SCS_MAJ, a high threshold, with a *Sp* of 0.93, can be accepted to classify correctly most or all of the uninfected udders as true negative, and most of

infected udder as true positive. However, these *Se* and *Sp* values are slightly larger than those reported by Bergonier et al. (2003a) for major pathogens. For SCS and SCS_MIN, where the prevalence's were equal to 0.36 and 0.33, respectively, the *Se* of threshold levels were slightly different (0.53 and 0.48, respectively) for the different prevalence's, according to what reported by Zweig and Campbell (1993), whereas *Sp* was equal to 0.86 in both cases.

The PPV and NPV indicated what fractions of positive and negative results respectively, were correctly classified. Predictive values are to be intended therefore as a way to interpret a given test result, rather than a performance measure. Because prevalence is incorporated, predictive values are not to be intended as properties of the test itself, but as the result of applying the test in one particular way (Zweig and Campbell, 1993). The PPV and NPV for each threshold value for the four diagnostic tests allowed classifying correctly the 69.0, 73.5, 72.6, and 91.0%, respectively of the samples. These values, corresponding to the efficiency described by Zweig and Campbell (1993), are similar to the values reported by Bergonier et al. (2003a) in ewe udders (71.1%).

Use of the LR has been discussed in several papers. In our study, it is interesting to highlight the relationship between LR and the ROC plot, being the former an expression of probability of test results, given the presence/absence of IMI. In general, a given *x* value of SCS with a given *y* value of LR for a given diagnostic test, indicates that this value is *y* times more likely to occur in an infected udder than in an uninfected udder. The LR can be calculated for a single test value (LR_p) or for results on one side of a particular threshold (LR+ or LR-). The LR+ for SCS equal to 3.33 for SCS_MAJ diagnostic test was 8.30, indicating that this SCS value was eight times higher in infected than in uninfected udders. However, this does not necessarily mean that a SCS value higher than 3.33 is eight times more likely to arise from infected udders than from uninfected udders. The LR for a single test value is generally considered the best summary measure of the observed test results (Choi, 1998). In our case, the $LR_{(x)}$ for the SCS_MAJ test value of 3.33 was 5.89. This value is the slope of tangent at point *x* on the ROC curve for a continuous test. Based on that, it is expected that using the ROC method, the exact value associated to an $LR_{(x)}$ of 1.00 (i.e., the point at which the diagnostic test becomes informative) can be found close to 3.00. It becomes therefore clear that only at cut-off levels greater than 3.00, SCS would have diagnostic value for the SCS_MAJ.

Our results show that the selected cut-off is different for SCS and SCS_MAJ. The SCS threshold could be used to detect any infection, in this case the cut-off value is lowered to maximize *Se*, whereas the SCS_MAJ threshold could be used to detect the IMI caused from major pathogens. In this case the threshold value is higher to

maximize *Sp*. However, PPV decreased from 0.69 to 0.37 as the prevalence decreases, but the LRs are independent of prevalence, and at a threshold of 2.81 SCS, a tested positive animal is 2.81 times more likely to be infected than an untested animal of the same flock, irrespective of the infection prevalence in the flock.

5.5 Conclusions

To evaluate the diagnostic capability of SCS and CMT to detect IMI, the ROC methodology has the great advantage of providing the most comprehensive description. The ROC curves allow a comparison between different diagnostic tests. The analysis of the curves provided useful information which enables to optimize the use of a test through targeted selection of threshold values for each diagnostic strategy considered. In our study, both the semi-parametric and non-parametric approaches showed that diagnostic capability of SCS and CMT was significantly different from chance and different between minor and major pathogens.

The results indicate that the CMT could only discriminate the udders infected from major pathogens. Nevertheless, in general SCS was the best indirect test for the bacteriological status of the udder. Considering the dynamic character of infection and cellular response, the definition of two thresholds (SCS_MIN and SCS_MAJ) within the same detection criterion of the IMI represents the optimal compromise to discriminate infected from uninfected udders. The overall accurate assessment of LRs, the *Se*, and *Sp*, corresponding to the threshold values of diagnostic tests (CMT, SCS, SCS_MIN, and SCS_MAJ) may allow using them as basic indicators to discriminate healthy from infected udders. However, further studies are needed to better define the economic appraisal of the mastitis cost in dairy sheep farms, given that the best OOP on the ROC curve will shift towards higher *Se* and lower *Sp*, when false positive costs decrease with respect to false negative costs, or when IMI prevalence increases.

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6

General discussion

6.1 Background

The objective of this thesis was to investigate genetic aspects of somatic cell count (SCC) and its relationship with milk production traits, longevity, and infection status of the udder. The aim of the investigation was to identify the best traits to include in a selection scheme to improve mastitis resistance in the Valle del Belice dairy sheep breed. In Chapter 2 heritabilities and correlations were estimated for somatic cell score (SCS) and milk production traits. These genetic parameters were, in general, in line with those reported in literature for dairy sheep breeds. Chapter 3 dealt with the effect of SCC on longevity, showing that an increase in SCC is associated with a reduction of longevity. In Chapter 4 heritabilities of SCS, according to whether the samples were bacteria negative or positive were estimated as well as the genetic correlations between bacteria negative and bacteria positive SCS and between bacteria negative SCS and the infection status. Moreover, the impact of imperfect sensitivity and specificity on variance component estimates was investigated. In Chapter 5, the diagnostic ability of SCC was evaluated by using the Receiver Operating Characteristic curves, in order to identify a SCC threshold that better discriminated healthy from infected udders.
In this chapter I will explore the opportunities to use SCS as indicator of mastitis in a selection scheme to improve mastitis resistance for the Valle del Belice dairy sheep breed. First I will present a short overview of recent developments in the dairy sheep production, which have stimulated selection on other traits in addition to milk production. Subsequently, the practical application of breeding value estimation for SCS will be discussed. This will be followed by a discussion of the correlations among SCS and other traits of interest and opportunities offered by alternative statistical methods to analyze SCS. Finally the prospects of improving mastitis resistance will be discussed.

6.2 Developments in dairy sheep production

Classically, farmers operating in dairy sheep production systems have considered milk yield as the major selection criterion. Most milk produced by sheep in Italy is processed into high quality cheese, often Protected Designation of Origin (PDO) cheese as laid down in European Union legislation. However, dairy sheep production in Europe is changing dramatically, as a consequence of globalization, reduction in production subsidies, increased emphasis on human health, food safety, animal welfare, and environmental legislation. In this novel context, there is a need to genetically improve dairy sheep for production (milk yield) and milk composition (fat and protein) in order to remain competitive in a global market. In

addition, more emphasis is needed to improve functional traits related to enhancing animal welfare, reducing production costs and increasing product quality and safety. This means that some traits are becoming more important for dairy sheep production, such as machine milking ability and udder morphology, resistance to diseases (e.g., mastitis, internal parasites, scrapie), milk nutritional value (fatty acid composition and bioactive peptides), reproduction traits, and lamb meat production. This includes additional emphasis on mastitis resistance, as this trait has an impact on animal welfare and farmers' income, mainly reflected by veterinary costs, decreased milk production and increased involuntary culling.
It is generally accepted that SCS is a good measure to select indirectly for mastitis resistance in dairy cattle. However, this is less clear in sheep, where the few studies (compared to cattle) available did not yield a consistent or unique picture.

6.3 Breeding value estimation and practical application

An important aspect of selection is the estimation of breeding values (EBVs) for the trait of interest. The EBVs can be used to select potential parents from the selection candidates. However, mastitis is not routinely recorded. It is, therefore, common practice to estimate breeding values for mastitis resistance based on information collected on correlated traits. In this thesis the following correlated traits were considered: SCS, longevity, and infection status. Some general conclusions regarding the best trait to select for can be drawn on the basis of the results obtained for the Valle del Belice breed. I reported that SCS can be high even when ewes are not infected (Chapter 4), suggesting that a healthy animal can wrongly be diagnosed as infected based on SCS. An alternative is to collect phenotypes for infection status, which gives a direct measure on whether an animal is infected or healthy. The bacteriological examination is indeed often considered to be the 'golden standard' for routine detection and identification of mastitis pathogens. On the other hand, however, it has to be taken into account that even good quality bacteriological or clinical mastitis data will have true Sensitivity (*Se*) and Specificity (*Sp*) values somewhat less than one, i.e., some cases will be missed and others will be misdiagnosed as infected when they are not. *Sp* and *Se* are important criteria in choice of traits to be recorded, as well as the heritability of the trait and the correlation with the target trait. Heritabilities estimated for SCS in this thesis are between 0.09 and 0.14, in the range reported in sheep literature (i.e., Baro et al., 1994; Mavrogenis et al., 1999; Barillet et al., 2001; Rupp et al., 2001; Hamann et al., 2004). The heritability for SCS is generally higher than the heritability estimate reported for the infection status (Chapter 4). When

only considering the heritability, this suggests that selection for SCS (as indicator of mastitis) is preferred over selection for infection status. However, before conclusions can be drawn correlations among traits should be considered. These will be discussed in section 6.4.

In the Valle del Belice breed, current selection is mainly practised on a "within farm" basis and based on own performance of ewes. In that situation, it is unlikely that selection for mastitis resistance will be successful, independent of the use of infection status or SCS. In this breed, therefore, the implementation of a structured breeding program needs to be realized in order to guarantee reliable pedigree recording and performance registration. This is essential in order to implement breeding value estimation.

When deciding upon the most appropriate trait to select for, one should also take into account the socio-cultural background of the farmers. Compared to collecting information on infection status, it is easier, cheaper, and less time-demanding for farmers to collect information on SCC, as this can be regularly recorded during milk recording at low cost. In that case, therefore, farmers would likely be more willing to cooperate because of the low costs and high frequency of recording. In contrast, samples for determining the infection status have to be collected with more attention than samples for SCC. The implementation of a protocol for collecting such samples by farmers might be difficult, requiring more commitment in order to ensure sufficient quality of sample collection. It might be therefore necessary, in this case, to have these samples collected by more qualified persons, with the obvious disadvantages of higher costs and additional time by the farmers.

Based on the above considerations, I conclude that with the support of a well structured organization taking responsibility for pedigree recording and breeding value estimation, selection for SCS in the Valle del Belice dairy sheep breed is more feasible and achievable than for other mastitis-related traits.

Selection for reduced SCS can help to reduce mastitis incidence. In this regard, preliminary results by Rupp et al. (2009) from a first-lactation survey in dairy sheep have provided evidence that selection based on SCS EBVs may help to improve resistance to clinical and subclinical mastitis: low SCS line animals showed a lower incidence of clinical mastitis; a lower prevalence of mammary abscesses and subclinical intramammary infections, especially at parturition; a better ability to recover from intramammary infections contracted during lactation; and lower SCS in bacteriologically positive samples. Our results suggest that animals with high SCS in bacteriologically negative samples are more liable to mastitis (Chapter 4), reinforcing the results of Rupp et al. (2009). Therefore, the approach of selecting animals for decreased SCS is justified and should help to reduce the prevalence of

mastitis even in the absence of knowledge about infection status. Collection of information on infection status at regular intervals will be valuable to monitor the actual mastitis incidence in the population to ensure that selection on correlated traits is still resulting in the desired improvement in udder health.
Estimated breeding values for SCS could be included in a total merit index with milk production traits and udder conformation traits. This will aim at improving both production efficiency and ewe functionality.

6.4 Correlations among SCS, infection status, and milk production traits

Although farmers select on several traits (e.g., udder conformation) based on own performance, milk yield is currently the most important selection criterion, for which phenotypic records are collected and breeding values are estimated in most dairy sheep breeds. Barillet (1997) suggested that the introduction of milk composition traits and/or functional traits (e.g., resistance to mastitis) as selection objectives should be addressed only when a breeding program has reached asymptotic annual genetic gain for milk yield. However, this ignores the correlated response in other economically important traits resulting from selection on milk production only. To quantify the likely correlated responses, it is important to determine the genetic correlations between different traits.
Most sheep milk is used for cheese production. Therefore, milk composition traits (fat and protein content) are also important because they affect the yield and the taste of cheese. The genetic correlation between milk yield and milk composition is unfavourable. There was, therefore, a need for introducing milk pricing and selection criteria including fat and protein percentages. Similarly, it is necessary to take SCC into account. In addition to relationship with mastitis resistance, it is important to highlight that SCC is also associated with cheese yield; an increase in SCC reduces cheese yield. In cattle, for example, it is common practice that dairy industries reduce milk prices if the SCC of the bulk tank milk exceeds certain thresholds; similar payment systems are becoming common in sheep as well (Legarra et al., 2007). Nevertheless, in the Italian system, the current payment system for sheep milk is still based on yield only, i.e., no price differentiation based on composition and SCC is made. It has been shown that SCC has a remarkable influence on the bulk milk composition and lactodynamographic parameters in the Valle del Belice breed (Giaccone et al., 2005). The milk pricing system needs to be changed to increase the emphasis on cheese making properties and udder health.

By including SCC in the payment system, farmers are likely to pay more attention to reducing SCC and thereby improving milk quality as well as udder health.
Unlike bovine mastitis, where SCS is unfavourably genetically correlated with milk yield, correlation estimates between milk production and mastitis traits are not consistent in sheep. Published genetic correlations between SCS and milk yield range from positive, i.e. antagonistic, to negative (Baro et al., 1994; El-Saied et al., 1998 and 1999; Barillet et al., 2001; Rupp et al., 2003). Genetic correlations between SCS and milk production traits estimated in this thesis were all positive (Chapter 2), indicating that selection for increased milk yield or fat and protein content would lead to higher SCS. However, the correlations are not close to 1, which implies that simultaneous improvement of milk yield and reduction of SCS is possible. Importantly, I found a genetic correlation between SCS in bacteria negative sheep and infection status of 0.51 (Chapter 4), suggesting that animals with lower SCS, assessed when apparently not infected, are genetically less likely to be infected (across all time points).
Although no correlations have been estimated between SCS and udder conformation traits in this breed so far, previous studies on other breeds showed favourable correlations between these traits (Legarra and Ugarte, 2005; Sechi et al., 2007). Results suggest that udders with what is perceived to be a good shape are less affected by sub-clinical mastitis. Pendulous and deep, poorly attached udders are difficult to milk and may cause sudden cluster falling, teat-end impacts, and subsequent bacterial infections (Bergonier et al., 2003). In addition, these udders are more prone to injuries. Based on these findings, I conclude that in the definition of a selection index for mastitis resistance for the Valle del Belice sheep breed, emphasis should be also given to udder conformation traits in addition to SCS.

6.5 Other statistical methods to analyze SCS

In using SCC as an indicator of mastitis, the dynamic nature of mastitis is often ignored in the statistical analysis. It has been reported that both clinical and subclinical mastitis cause deviations from a typical curve of SCC (e.g., de Haas et al., 2004). In this respect, use of individual SCC test-day records is an improvement compared to the average of SCC records collected during the lactation. However, Urioste et al. (2010) reported that the use of test-day SCC can still make it difficult to identify short-duration infections, because SCC is often only recorded at approximately monthly intervals. Therefore, Urioste et al. (2010) suggested exploring alternative traits derived from the SCC curve (i.e., traits designed to

capture SCC base levels and variation along the curve, time and level of infection, and time of recovery). Ideally, these alternative traits should be able to accommodate sudden and drastic changes in SCC, which in turn will improve the diagnosis of mastitis and hence increase genetic progress in mastitis resistance. In my opinion, however, there are limitations to the use of these alternative traits on commercial farms. If it is true that the shortcoming of SCC is that it is only recorded monthly, making it difficult to identify short-duration infections, then these alternative traits are unlikely to contain more information as they are based and designed on the same original information (i.e., test-day SCC). Moreover, ewes are milked (and, therefore, SCC records available) only once lambs are fully weaned, which can lead to an early misclassification of healthy and infected animals. Therefore, these alternative traits can probably be explored, used and better exploited on experimental farms, where the SCC records can be collected more frequently.

In genetic evaluation of SCS, information collected on healthy and infected animals is treated equally. However, several authors suggested that, in cattle, SCS in healthy and infected animals, are different traits (Detilleux and Leroy, 2000; Boettcher et al., 2007; Madsen et al., 2008). This was also confirmed in sheep in this thesis (Chapter 4), in which we showed that SCS in healthy and infected animals can indeed be considered as different traits, with different heritabilities, and with a genetic correlation between bacteria negative and bacteria positive SCS of 0.62. Whilst this genetic correlation is moderately positive, it is significantly less than unity, suggesting that bacteria negative and bacteria positive SCS are not the same trait. Genetic evaluation of SCS can be improved when this non-unity genetic correlation is taken into account. In most countries, however, cases of mastitis are not routinely recorded in a systematic manner. The lack of information on the infection status is a limitation in selecting directly for mastitis resistance. It implies that when using SCS as indicator of mastitis, no distinction can be made between SCS data from infected and uninfected animals.

When information on infection status is not available, SCS may be regarded as a mixture of observations from animals with unknown health status, i.e., with and without mastitis. Mastitis infection would produce a deviation from the SCS baseline level, i.e., an observed test-day SCS can be regarded as resulting from effects of a baseline SCS (a continuous trait) and a deviation caused by a binary process (healthy or infected). Detilleux and Leroy (2000) have shown that a finite mixture model can account for these differences and can represent a latent structure in a set of data whereby observations may belong to one of several distributions, possibly differing in mean, variance, and even the type of distribution

(McLachlan and Peel, 2000). Recently, ten Napel et al. (2009) have shown that there is indeed evidence in the distributions of SCC values that some SCC are an indication of an infected udder or quarter and others are indicative of a response to infection or a recovery from an infection. In particular, these authors highlighted that describing the observed distribution by a mixture of 4 normal and 1 exponential distributions provides an opportunity to distinguish uninfected animals from animals infected with minor or major pathogens.

Using mixture models, therefore, selection for reduced mastitis incidence may be based on the probability of mastitis given SCS, rather than selection for lowest possible SCS. Recent research has also been done to extend the ideas of Detilleux and Leroy (2000) to develop a finite mixture model for SCS using a Bayesian approach (e.g., Ødegård et al., 2003; Gianola et al., 2004; Boettcher et al., 2007).

Regarding analysis of SCS data, Boettcher et al. (2007) have tested four different mixture models and all were found to be more appropriate for analysis of these data than the standard linear model. Moreover, although correlations of ca. 0.90 were found between breeding values from mixture and linear models, changes in ranking of the higher ranked sires were reported, showing that practical benefits would be realized with the adoption of a mixture model for genetic evaluation. In conclusion, mixture models are potentially useful and a good alternative for analysis of SCS data. However, although biologically speaking their use makes sense, they require a good data recording. Moreover, these models might be difficult to implement in practical breeding values estimation because of computational limitations. Based on the above considerations, I would not recommend the use of these models for the Valle del Belice breed.

6.6 Actual situation and prospects for improvement

Technical and infrastructural related issues are the greatest bottlenecks in genetic improvement programs for Italian sheep farming systems. Small flock sizes, poor pedigree and performance recording, lack of clear breeding goals, lack of or poor infrastructures: these factors contribute to the low participation of farmers in breeding schemes, which in turn makes achieving within-breed genetic improvement highly challenging.

Whereas artificial insemination (AI) is a common reproductive technique in dairy cattle, in dairy sheep its application is limited to experimental farms. Due to the low use of AI, the diffusion rate of a ram is from 100 to 1,000 times lower than that of a bull (Carta et al., 2009). A major problem in using AI in sheep is the low conception rate, i.e., the AI conception rates using fresh or frozen semen are

approximately 55% and 25%, respectively. The anatomical structure of the ewe's cervix makes the penetration of the cervix during AI difficult, which contributes to the low conception rate. If semen could be deposited in the uterus as is the case with cattle, the conception rate would improve to the point where AI would be attractive on sheep commercial farms. When AI is used for sheep, it is generally laparoscopic, and it has been shown that conception rates using frozen semen range from 50% to 80% when a skilled technician uses a laparoscopic insemination technique to place the semen directly into the ewe's uterus. However, surgical insemination is obviously a veterinary procedure, it is expensive and it has been criticised on welfare grounds. Taken together, the low conception rates have severely restricted the use of AI in sheep.

The limited use of AI reduces the progeny group size of rams and is in general associated with poor pedigree recording, which negatively affects the accuracy of breeding value estimates (Van Vleck, 1970; Lee and Pollak, 1997). Many flocks rely on a few males, and it is not possible to know with certainty which ram is the sire of an animal. In dairy cattle, it has been reported that paternity errors can reach up to 20% of registered animals (Ron et al., 1996) and this percentage is probably even higher in sheep, drastically reducing the genetic gain and the success of breeding programs. To overcome this problem, it has been suggested to Valle del Belice farmers to manage natural mating by grouping ewes with a single ram (i.e., mating group) during the reproduction period. This management strategy would make it easier to determine the correct sire of a lamb based on the lambing date. However, the poor infrastructures on the farms in general do not allow the implementation of these strategies. As an alternative, it may be possible to use DNA testing for pedigree verification or pedigree assignment in cases of unrecorded mating or the use of multiple sires. Procedures have been already developed for many species, including dogs (DeNise et al., 2004), horses (Tozaki et al., 2001; Seyedabadi et al., 2006), cattle (Van Eenennaam et al., 2007), goats and sheep (Glowatzki-Mullis et al., 2007).

Another problem encountered in genetic evaluation of Valle del Belice flocks is the poor genetic connections between flocks, which results from the limited exchange of rams between farms. This is could be overcome by AI but as discussed early the uptake of AI is low. This implies that improvements in genetic connections need to come from exchanging rams between farms. However, farmers do not see it as favourable to exchange rams between flocks, as they usually think they have the best individuals.

An alternative would be to implement a selection scheme for the Valle del Belice sheep based on the pyramid management of the population, which is nowadays

considered the most efficient selection scheme for local dairy sheep (Barillet, 1997). In this scheme, the nucleus flocks are at the top of the breeding pyramid. In these flocks, pedigree and milk recording are implemented, and breeding value estimations are carried out to generate genetic progress in these flocks. The genetic progress would be then disseminated to commercial flocks through AI or natural-mating rams originated from nucleus flocks. A potential problem in the implementation of this scheme is that farmers would need to be convinced of the superior quality of the rams from the nucleus flock.

However, I believe that farmers will be willing to cooperate in such a scheme once they experience the quality of the breeding products. I expect that this will be especially the case for young farmers. It would even be easier to realize such a scheme if it were technically or financially supported by the Regional Government or the University. The support by such an Institution would reassure farmers, who sometimes just need to feel that their interests are taken into account.

When implementing a nucleus breeding scheme, an important issue is genotype by environment (GxE) interaction. GxE interactions would reduce the benefits for commercial farmers of genetic progress generated in the nucleus. One of the methods used to quantify GxE is estimation of genetic correlations (r_g) between traits measured in different environments. When r_g between the phenotypic values of the same trait expressed in different environments is high, i.e., equal or close to 1, then there is no GxE (Robertson, 1959). On the other hand, low r_g values indicate a GxE, i.e., phenotypes expressed in different environments are expressions of different traits. Mulder and Bijma (2005) estimated that a r_g of 0.80 between two environments results in 20% less genetic gain for a trait in dairy cattle, when breeding stock are selected in another environment. Moreover, Mulder et al. (2006) showed that in dairy cattle, when r_g between environments are higher than 0.50 to 0.70, a single breeding program with progeny testing bulls in different environments would be optimal to breed for general adaptability. However, when r_g between environments are lower than 0.50 to 0.70, environment-specific breeding programs are necessary to breed for special adaptability. Therefore, to realize a pyramid selection scheme for the Valle del Belice breed, it would be important to make sure that the environment at nucleus flocks is comparable to that at commercial farms.

Concerning diseases and disease resistance, quantifying and accounting for the impact of environmental factors is an important part of identifying and measuring true host genetic variation in resistance to the disease under study. There is a risk of biases in genetic parameter estimates and lost opportunities for identifying individuals with extreme genetic risk when these environmental factors are not

correctly taken into account (Bishop and Woolliams, 2010). It is therefore necessary to determine the "optimal exposure level" in order to select for mastitis resistance. Of course it would not be good to have all animals to infected; however, on the other hand, if no animals are affected then there is no information upon which to base selection. It is important to realize that a lack of exposure simply means that individuals do not have the opportunity to express their genetic merit for resistance, with potentially highly susceptible individuals being (wrongly) classified as resistant simply because they are healthy (Bishop and Woolliams, 2010). These authors have demonstrated that whilst true presence/absence of a disease, given exposure to infection, will be largely a function of the immune response, the actual prevalence of the disease and the estimable genetic variation between animals will be influenced by variable exposure and the sensitivity of diagnosis.

In implementing a breeding scheme for mastitis resistance, it has to be taken into account that measurements of phenotypic indicators for mastitis resistance are time and labour intensive. Therefore, the use of genetic markers to better exploit the phenotypic information through genomic selection (GS) (Goddard and Hayes, 2007) or to indicate resistance or susceptibility to mastitis is an attractive proposition. At present, however, the available literature on GS and molecular markers for mastitis resistance mainly refer to dairy cattle (e.g., Klungland et al., 2001; Boichard et al., 2003; Schulman et al., 2004). In sheep, quantitative trait loci (QTL) influencing SCS have recently been detected (i.e., Gutiérrez-Gil et al., 2007; Raadsma et al., 2009).

There is widespread excitement about the potential for GS to provide new approaches for the improvement of sustainability traits in Holstein dairy cows, and many breeding programs worldwide have already implemented GS. However, it is important to recognize that is not obvious how GS can be implemented in small ruminant species. An important limitation of applying GS to sheep is that a reference population of considerable size would be required. In dairy cattle, for example, reference populations of over 4,000 progeny tested young bulls are available, and this scale would be difficult to achieve in sheep. However, nowadays, thank to the development of high-density SNP arrays with tens of thousands of genetic markers spread across the genome, research is moving to the direction of GS in sheep as well, as such arrays have also proved very powerful with even small numbers of animals.

6.7 Conclusions

The results reported in this thesis suggest that it is possible to select for improved mastitis resistance in the Valle del Belice dairy sheep breed. In this chapter, I have highlighted a number of elements that need to be considered when setting up a breeding program for mastitis resistance. Besides the importance of knowledge of both genetic and environmental aspects of mastitis resistance, I have stressed the need for having a strong and well structured organization to implement and support the program.

The heritabilities of the traits of interest, either SCS or infection status, are quite low. Therefore, it is unlikely that selection for mastitis resistance by the farmers on their own will be successful. However there is good prospect for genetic improvement at farm level when reliable pedigree and performance recording is implemented across flocks and combined with breeding value estimation. This system requires cooperation between farmers and technical support from an independent organisation. A cooperative breeding program which exploits a nucleus structure in combination with genetic evaluation will lead to an increased contribution of the local sheep production sector to the Sicilian economy.

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Summary

Mastitis is an inflammation of the udder, generally caused by bacteria, and it leads to economic loss, mainly consisting of discarded milk, reduced milk production and reduced milk quality, and increased health costs. In case susceptibility to mastitis is heritable, genetic selection can be used to increase resistance to mastitis in order to reduce the incidence of the disease. Selecting for increased genetic resistance to mastitis can be done directly or indirectly. Direct selection corresponds to the diagnosis of the infection, whereas the indirect selection corresponds to a prediction of the bacteriological status of the udder based on traits related to the infection (e.g. inflammatory parameters). Simple, indirect methods have been widely applied, based on the evaluation of the degree of inflammation or of internal mammary lesions, and among them, the most frequently used to detect mastitis is milk somatic cell count (SCC) or somatic cell score (SCS). This thesis focuses on the genetic parameters of SCS as indicator of mastitis, and on the possibilities of using this trait in selection for mastitis resistance in the Valle del Belice dairy sheep.

The objectives of this thesis were to analyze genetic aspects of SCS in Valle del Belice dairy sheep, in order to study the use of SCS data in genetic selection for mastitis resistance. In Chapter 1, mastitis and SCS are defined and introduced. Chapter 2 deals with the estimation of genetic parameters (i.e., heritabilities and correlations) for SCS and milk production traits in primiparous Valle del Belice ewes. In Chapter 3, the level of SCC is included in a survival analysis in order to evaluate the effect of SCC on functional longevity. In Chapter 4, the genetic parameters for infection status and SCS, according to whether the samples were bacteria negative or positive are reported. Moreover, the impact of imperfect sensitivity and specificity on variance component estimates was investigated. In Chapter 5, the diagnostic ability of SCC was evaluated by using the Receiver-Operating Characteristic curves, in order to identify a SCC threshold that discriminates healthy from infected udders in sheep. In the General Discussion, the opportunities to use SCS as indicator of mastitis in a selection scheme to improve mastitis resistance for the Valle del Belice dairy sheep breed are explored.

In Chapter 2, genetic parameters were estimated for SCS and milk production traits in the Valle del Belice dairy sheep breed, using a repeatability test-day animal model. Heritability estimates were low and ranged from 0.09 to 0.14 for milk, fat and protein yields and contents. For SCS, the heritability of 0.14 was relatively high. The repeatabilities were moderate and ranged from 0.29 to 0.47. Flock-test-day explained a large proportion of the variation for milk production traits but did not

have a big effect on SCS. The analyses also showed that SCS is genetically positively correlated (range 0.16 to 0.31) to milk, fat and protein yields and contents. Therefore, selection for increased milk production will also increase SCS. However, correlations are not extreme, so simultaneous improvement for milk yield and SCS seems possible.

In Chapter 3, the effect of SCC on functional longevity in Valle del Belice dairy sheep is investigated. An increase in SCC was associated with an increase in culling rate. These results demonstrate that elevated SCC play an indirect role in the culling decisions of Valle del Belice dairy sheep farmers, although, at present, farmers do not directly select for reduced SCC. The heritability estimate for functional longevity was 7% on the logarithmic scale and 11% on the real scale. The proportion of additive genetic variation estimated for functional longevity in Valle del Belice ewes, therefore, indicates that it may be possible to improve productive life by genetic selection.

In Chapter 4, the genetic parameters for infection status and SCS, according to whether the samples were bacteria negative or positive were investigated. Moreover, the impact of imperfect diagnosis of infection on variance component estimates was evaluated. The heritability of SCS was 0.10 for bacteria negative samples, 0.03 for SCS of bacteria positive samples, and 0.09 for infection status, on the liability scale. The genetic correlation between SCS of bacteria negative samples and SCS in bacteria positive samples was 0.62 and suggests that they may be genetically different traits, confirming that SCC from healthy and infected animals should be analyzed separately. Moreover, a positive genetic correlation between SCS in bacteria negative milk samples and liability to mastitis was found, suggesting that selecting animals for decreased SCS will help to reduce the prevalence of mastitis. The results also showed that imperfect diagnosis of infection has an impact on estimated genetic parameters, which may reduce the efficiency of selection strategies aiming at distinguishing between bacteria negative and bacteria positive SCS.

In Chapter 5, the diagnostic capability of SCS and California Mastitis Test (CMT) to detect intramammary infections was investigated using the Receiver-Operating Characteristic curves methodology. Three different SCS traits were considered: SCS for the whole sample (i.e., considering uninfected and infected glands), SCS for minor pathogens (SCS_MIN - i.e., considering uninfected and infected by minor pathogens glands), and SCS for major pathogens (SCS_MAJ - i.e., considering uninfected and infected by major pathogens glands). The results indicate that the four diagnostic tests (CMT, SCS, SCS_MIN, and SCS_MAJ) allowed correctly classifying 69.0, 73.5, 72.6 and 91% of infected udders, respectively. Moreover, the

results indicate that the CMT can only discriminate the udders infected from major pathogens. Nevertheless, in general SCS was the best indirect test for the bacteriological status of the udder.

The final chapter explores and discusses the opportunities to use SCS as indicator of mastitis in a selection scheme to improve mastitis resistance for the Valle del Belice dairy sheep breed. A number of elements that need to be considered in this process have been highlighted: i.e., the practical application of breeding values estimation for SCS, the correlations among SCS and other traits of interest, the opportunities offered by alternative statistical methods to analyze SCS. In the Valle del Belice breed, where the current selection is mainly practised on a "within farm" basis and based on own performance of ewes, it is unlikely that selection for mastitis resistance is successful, independent of the use of infection status or SCS. However, it was highlighted that with the support of a well structured organization taking responsibility for pedigree recording and breeding values estimation, selection for SCC is better achievable than other mastitis-related traits for the Valle del Belice dairy sheep breed.

Samenvatting

Mastitis is een ontsteking van de uier, meestal veroorzaakt door bacteriën. Mastitis leidt tot economische schade, voornamelijk omdat de melk niet langer geschikt is voor humane consumptie, maar ook door verminderde melkproductie, verminderde kwaliteit van de melk, en hogere veterinaire kosten. Wanneer gevoeligheid voor mastitis erfelijk is dan kan genetische selectie worden gebruikt om de weerstand tegen mastitis te verhogen om daarmee de incidentie van de ziekte te verminderen. Genetische selectie voor een hogere resistentie tegen mastitis kan door middel van directe of indirecte selectie. Directe selectie is gebaseerd op daadwerkelijk vastgestelde infecties (bacteriologisch onderzoek) terwijl indirecte selectie gebaseerd is op voorspelling van de bacteriologische status van de uier op eigenschappen die gerelateerd zijn aan de infectie (bijv. inflammatoire parameters). Eenvoudige indirecte methoden om mastitis te detecteren worden op grote schaal toegepast en zijn onder andere gebaseerd op het scoren van de mate van ontsteking. De meest gebruikte indirecte en eenvoudige methode om mastitis te detecteren is het melkcelgetal (SCC) of de celgetalscore (SCS) . Dit proefschrift richt zich op genetische parameters voor SCC en SCS, en op de mogelijkheden om door middel van selectie op SCS een verhoogde weerstand tegen mastitis infecties in de Valle del Belice melkschapen te bewerkstelligen.

Doelstellingen van dit proefschrift waren om de genetische aspecten van SCS in Valle del Belice melkschapen te analyseren. Dit met het oog op het gebruik van SCS in de genetische selectie voor mastitis resistentie. In hoofdstuk 1 worden mastitis en SCS geïntroduceerd. Hoofdstuk 2 handelt over het schatten van genetische parameters (dat wil zeggen erfelijkheid en correlaties) voor SCS en melkproductiekenmerken in eerste pariteits Valle del Belice ooien. In hoofdstuk 3 wordt het effect van SCC op functionele levensduur geëvalueerd middels een survival analyse. In hoofdstuk 4 worden de genetische parameters voor bacteriologische status en voor SCS geschat. Voor SCS werden de genetische parameters afzonderlijk geschat voor bacteriologisch negatieve en bacteriologisch positieve monsters. Bovendien werden de gevoeligheid van variantie component schattingen voor sensitiviteit en specificiteit van de bacteriologische test onderzocht. Om op grond van het celgetal onderscheid te kunnen maken tussen een gezond en een geïnfecteerd uier is in hoofdstuk 5 door middel van ROC curves het diagnostische vermogen van SCS geëvalueerd. In de algemene discussie zijn de mogelijkheden onderzocht om middels selectie op SCS een verhoogde weerstand tegen mastitis in Valle del Belice melkschapen te bewerkstelligen.

In hoofdstuk 2, zijn geschatte genetische parameters voor SCS en melkproductiekenmerken in Valle del Belice melkschapen beschreven. Hierbij is gebruik gemaakt van een test-dag model. Erfelijkheidsgraadschattingen waren laag en bedroegen 0,09 tot 0,14 voor kilogrammen melk, vet en eiwit en voor melksamenstelling (vet% en eiwit%). Voor SCS was de erfelijkheidsgraad 0,14 en in vergelijking tot de melkproductiekenmerken relatief hoog. De herhaalbaarheden waren matig en varieerden van 0,29 tot 0,47. Het bedrijf-testdag effect verklaarde een groot deel van de variatie in melkproductiekenmerken, maar had geen grote invloed op de variatie in SCS. Uit de analyses bleek ook dat SCS genetisch positief gecorreleerd was (variërend van 0,16 tot 0,31) met melk, vet en eiwit opbrengst en melksamenstelling (vet% en eiwit%). Daarom zal selectie voor verhoogde melkproductie naar verwachting leiden tot hogere SCS. Echter, de correlaties waren niet extreem, zodat gelijktijdige verbetering van de melkproductiekenmerken en SCS mogelijk lijkt.

In hoofdstuk 3 is het effect van SCC op de functionele levensduur van Valle del Belice melkschapen onderzocht. Een hogere SCC was geassocieerd met een hogere uitval. Hoewel Valle del Belice schapenhouders op dit moment niet selecteren op een lager celgetal tonen deze resultaten aan dat verhoogde SCC een indirecte rol spelen bij het besluit om schapen af te voeren. De erfelijkheidsgraadschatting voor functionele levensduur was 7% op de logaritmische schaal en de schatting van de effectieve erfelijkheidsgraad was 11%. De geschatte additief genetische variatie voor functionele levensduur in Valle del Belice ooien suggereert dat het mogelijk is om de productieve levensduur te verbeteren door middel van genetische selectie.

In hoofdstuk 4 zijn de genetische parameters voor infectie status en SCS geschat waarbij onderscheid is gemaakt tussen bacteriologisch positieve en bacteriologisch negatieve monsters. Bovendien is de impact van de nauwkeurigheid van de diagnose van infectie op de variantiecomponent schattingen geëvalueerd. De erfelijkheid was 0,10 voor SCS van bacteriologisch negatieve monsters, 0,03 voor SCS van bacteriologisch positieve monsters, en 0,09 voor infectie status (op de onderliggende schaal). De genetische correlatie tussen SCS van bacteriologisch negatieve monsters en SCS van bacteriologisch positieve monsters was 0,62 en suggereert dat dit genetisch verschillende eigenschappen zijn. Dit bevestigt dat SCS van gezonde en besmette dieren afzonderlijk moeten worden geanalyseerd. Ook werd een positieve genetische correlatie gevonden tussen SCS in bacteriologisch negatieve melkmonsters en infectie status. Dit wijst erop dat het selecteren van dieren voor lager SCS zal helpen om de incidentie van mastitis te verminderen. De resultaten toonden ook aan dat een gebrekkige diagnose van infectie gevolgen had voor de geschatte genetische parameters. Dit kan effect hebben op de efficiëntie

van selectie strategieën die als doel hebben om de weerstand tegen mastitis te verhogen.

In hoofdstuk 5 zijn de diagnostische mogelijkheden van SCS en de Californië Mastitis Test (CMT) om intra-mammaire infecties te detecteren onderzocht met behulp ROC curves. In het onderzoek zijn drie verschillende SCS eigenschappen vergeleken: SCS voor de gehele steekproef (dat wil zeggen geïnfecteerde en niet geïnfecteerde uiers), SCS voor ziekteverwekkers met geringe pathogeniteit (SCS_MIN – waarbij onderscheid is gemaakt tussen uiers met en zonder ziekteverwekkers met geringe pathogeniteit), en SCS voor belangrijke pathogenen (SCS_MAJ – waarbij onderscheid is gemaakt tussen uiers met en zonder belangrijke pathogenen). De resultaten gaven aan dat de vier diagnostische tests (CMT, SCS, SCS_MIN en SCS_MAJ) respectievelijk 69,0, 73,5, 72,6 en 91% van de besmette uiers correct classificeerden. Bovendien lieten de resultaten zien dat CMT alleen de uiers die geïnfecteerd waren met de belangrijke pathogenen kan identificeren. In het algemeen is SCS de beste indirecte test voor de bacteriologische status van de uier.

Het laatste hoofdstuk onderzoekt en bediscussieerd de mogelijkheden om SCS te gebruiken als selectiecriterium ter verbetering van de mastitis weerstand in Valle del Belice melkschapen. Een aantal elementen die van belang zijn worden besproken: de praktische implementatie van fokwaardeschatting voor SCS, de correlaties tussen SCS en andere belangrijke kenmerken en de mogelijkheden die geboden worden door alternatieve statistische methoden om SCS te analyseren. In het Valle del Belice ras, waar de huidige selectie met name binnen het bedrijf plaatsvindt en is gebaseerd op de eigen prestaties van ooien, is het onwaarschijnlijk dat selectie op weerstand tegen mastitis veel zal opleveren, ongeacht het gebruik van infectie status of SCS. Echter, met de steun van een goed gestructureerde organisatie die de verantwoordelijkheid neemt voor afstammingsregistratie en fokwaardeschatting is selectie op SCC praktisch beter uitvoerbaar dan selectie op andere aan mastitis-gerelateerde kenmerken.

Curriculum vitae

Valentina Riggio was born in Palermo (Italy). She graduated in Agriculture with a degree in Animal Science at University of Palermo (Italy) in 2002. In 2004, she spent a 6 months study period at The Roslin Institute (Scotland) with a fellowship from University of Palermo. From 2004 to 2007 she worked on a PhD entitled “Analysis methods of binary traits: Applications in sheep production” at University of Reggio Calabria (Italy). In 2007, she started a sandwich PhD between University of Palermo and Wageningen University. As part of this she spent a 2 month period at the University of Madison-Wisconsin (USA) in 2007 and a 5 month period at the Roslin Institute (Scotland). Since November 2010, she is appointed as a post-doc at the Roslin Institute (Scotland), on a European Project on nematode resistance in sheep.

List of publications

Peer-reviewed papers

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Riggio, V., O. Matika, R. Pong-Wong, and S. Bishop, 2012. Comparison of genome-wide association and regional heritability mapping approaches to identify loci underlying faecal egg count variation in Blackface lambs. Accepted as poster presentation for the 4th International Conference on Quantitative Genetics, Edinburgh, 17-22 June, 2012.

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Thank you!

Valentina

Training and Supervision Plan		**Graduate School WIAS**	
Name PhD student	Valentina Riggio		
Group	Animal Breeding and Genomics Centre/University of Palermo		
Daily supervisor(s)	Prof. B. Portolano and Dr. H. Bovenhuis		
Supervisor(s)	Prof. J.A.M. van Arendonk		
The Basic Package		**year**	**credits**
WIAS Introduction Course (mandatory, 1.5 credits)		2008	1.5
Course on philosophy of science and/or ethics (mandatory, 1.5 credits)		2008	1.5
Subtotal			**3.0**
Scientific Exposure			
International conferences *(minimum 3 credits)*			
56th Annual Meeting of the EAAP, Uppsala, Sweden (4 days from 5 to 8 June 2005)		2005	1.2
8th World Congress on Genetic Applied to Livestock Production, Belo Horizonte, Brazil (6 days from 13 to 18 August 2006)		2006	1.8
58th Annual Meeting of the EAAP, Dublin, Ireland (4 days from 26 to 29 August 2007)		2007	1.2
59th Annual Meeting of the EAAP, Vilnius, Lithuania (4 days from 24 to 27 August 2008)		2008	1.2
9th World Congress on Genetic Applied to Livestock Production, Leipzig, Germany (6 days from 1 to 6 August 2010)		2010	1.8
61th Annual Meeting of the EAAP, Heraklion, Crete, Greece (5 days from 23 to 27 August 2010)		2010	1.5
15th QTL-MAS Workshop, Rennes, France (two days from 19 to 20 May 2011)		2011	0.6
Seminars and workshops			
WIAS Science day		2005	0.3
Animal Genetics Workshop at University of Palermo		2008	0.3
Presentations *(minimum 4 original presentations of which at least 1 oral, 1 credit each)*			
56th Annual Meeting of the EAAP, Uppsala, Sweden (oral)		2005	1.0
16th Congress of the ASPA, Turin, Italy (oral)		2005	1.0
8th World Congress on Genetic Applied to Livestock Production, Belo Horizonte, Brazil (oral)		2006	1.0
17th Congress of the ASPA, Alghero, Italy (oral)		2007	1.0
58th Annual Meeting of the EAAP, Dublin, Ireland (oral)		2007	1.0
9th World Congress on Genetic Applied to Livestock Production, Leipzig, Germany (oral)		2010	1.0
15th QTL-MAS Workshop, Rennes, France (oral)		2011	1.0
Subtotal			**16.9**
In-Depth Studies			
Disciplinary and interdisciplinary courses			
Course "How to breed livestock in Sicily?", Palermo, Italy 06 October-3 November (44 hrs) Lecturer: Dr. Egbert Knol		2006	1.5
Course "Survival analysis applied to Animal Brreding and Epidemiology", Bad Bramstedt Germany, 12-16 March 2007 - Lecturer: Dr. Vincent Ducrocq		2007	1.5
Course "Principles of Breeding Scheme Design", Palermo, Italy 20 April-8 June (44 hrs) Lecturer: Dr. Richard Spelman		2007	1.5
WIAS course "Fortran 95 for Animal Breeders" 11-15 June		2007	1.5
WIAS course "Understanding Genotype Environment Interactions: The biological basis for improved management and selection strategies" 25-29 June		2007	2.0
WIAS course "Linear Models in Animal Breeding" 2-6 July		2007	1.5
Course "Genomic Selection in Livestock", Davos, Switzerland 20-24 June Lecturers: Prof. Dr. R.L. Fernando and Prof. Dr. D.J. Garrick		2011	1.5
Advanced statistics courses *(optional)*			
WIAS course "Introduction to R for Statistical Analysis"		2008	0.6
Subtotal			**11.6**
Professional Skills Support Courses			
Course Techniques for Scientific Writing (advised)		2008	1.2
Project- and Time Management		2008	1.5
Interpersonal communication for PhD students		2008	0.6
Subtotal			**3.3**
Research Skills Training			
Preparing own PhD research proposal (maximum 6 credits)		2007	6.0
External training period Madison, Wisconsin 2 months		2007	2.0
External training period Edinburgh, Scotland 5 months		2008	2.0
Subtotal			**10.0**

Didactic Skills Training		
Supervising theses		
Luisa De Simone MSc major thesis	2007	2.0
Subtotal		**2.0**
Management Skills Training		
Organisation of seminars and courses		
Animal Genetics Workshop at University of Palermo on January 25 2008	2008	2.0
Subtotal		**2.0**
Education and Training Total		**48.8**

Colophon

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