Predisposing factors and control of bacterial mastitis in dairy ewes

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ABSTRACT. Bacterial mastitis is a major health problem in dairy sheep worldwide. It is associated with reduced milk yield and occasionally involuntary culling of affected ewes, as well as insufficient growth and mortality of lambs. In general, the incidence of clinical mastitis in ewes during lactation is lower than 5%. However, the prevalence of subclinical mastitis is variable ranging from 10-30% or more. In clinical cases of bacterial intramammary infections, *Staphylococcus aureus* is dominant. In the case of subclinical mastitis the prevailing isolates are Coagulase-Negative Staphylococci (CNS). Moreover, predisposing factors related to the environment, genetics, udder morphology and nutrition, are likely to contribute to the occurrence of mastitis. Hence, control of the latter factors and bacteriological contamination during milking are the main preventive measures. The notion is that detection of the ovine genome regions involved in mastitis resistance will also facilitate the most effective control of mastitis in flock level. Appropriate genetic selection, together with the implementation of preventive measures, could reduce the negative consequences of bacterial mastitis.

Keywords: dairy ewes, genetic selection, intramammary infections, mastitis, risk factors
INTRODUCTION

Ovine mastitis can be specified as the inflammation of the ewe’s udder (Schalm et al., 1971). Two forms of mastitis have been recognized according to its clinical features; clinical and subclinical mastitis. Clinical mastitis may be followed both by clinical findings (swelling, heat, redness, hardness or pain in the udder) and by abnormalities in milk (watery appearance, flakes, clots, or pus in the milk) (Bergonier and Berthelot, 2003; Olechnowicz and Jaskowski, 2014). On the contrary, in subclinical mastitis there are no obvious clinical findings, but, quantitative and qualitative changes in milk composition are usually observed (Bergonier and Berthelot, 2003; Fragkou et al., 2014).

Various causative factors are involved in the pathogenesis of ovine mastitis, with the most common being bacterial and viral agents. Among them, Staphylococci, Mannheimia haemolytica, Streptococci and Lentiviruses seem to be the most prevalent pathogens associated with the occurrence of mastitis (Bergonier and Berthelot, 2003; Turin et al., 2005; Contreras et al., 2007), whereas, less often, several other pathogens have been found to be associated with intramammary infections. The aforementioned pathogens can be broadly classified as contagious and environmental based on their virulence and origin. In any case and irrespectively of the causative pathogen, many environmental and genetic factors have been found to predispose to mastitis in sheep (Larsgard and Vaabenoe, 1993).

Controlling for the risk factors and eliminating the causative agents of mastitis is crucial for modern dairy sheep farms, both for maintaining a high health and welfare status of dairy sheep and for the elimination of economic losses due to mastitis (Radostits et al., 2000; Barillet et al., 2001; Conington et al., 2008). Therefore, the last decades, ovine mastitis has been recognized as an issue of major concern for dairy sheep industry, which has resulted in an increasing body of research (Contreras et al., 2007). For example, recently, a project funded by the European Commission has been focused on the research of sustainable management on small ruminants’ health problems, including mastitis and under the acronym 3SR-Sustainable Solutions for Small Ruminants.

However, extrapolating data from bovine mastitis is still the most common practice when approaching ovine mastitis and its control. Considering the noticeable differences between the two species, a more species-specific approach needs to be adopted. In the case of dairy ewes, current breeding programs focus almost exclusively on selection for high milk yield potential, which, com-
bined with the implemented intensive management schemes has lead to an increased incidence risk of intramammary infections and mastitis. Hence, the objective of this paper was to review the knowledge regarding risk factors and control measures against bacterial mastitis in dairy sheep.

**Etiology**

According to the available literature, Staphylococci are the major etiological agents of mastitis in dairy sheep flocks. Particularly, coagulase-negative staphylococci (CNS) and *Staphylococcus aureus* are the most commonly isolated bacteria in cases of subclinical and clinical mastitis, respectively (Bergonier et al., 2003; Leitner et al., 2004; Contreras et al., 2007). On the other hand, in meat and wool producing breeds of sheep, *Mannheimia haemolytica* is the most commonly isolated bacterium from mastitis cases (Mavrogianni et al., 2007, Omaleki et al., 2010). In a descending order, *Streptococcus* spp. (Marogna et al., 2010), *Corynebacterium* spp. (Spanu et al., 2011), Enterobacteriaceae (Fthenakis et al., 2004; Mork et al., 2007), *Listeria monocytogenes* (Brugère-Picoux, 2008) and other bacteria have been found to be related with mastitis in dairy ewes (Table 1).

**Epidemiology**

The annual incidence of clinical mastitis is generally lower than 5% (Bergonier et al., 2003; Contreras et al., 2007). However, some authors have reported outbreaks where the incidence of clinical mastitis ranged from 30% to 50% (Lafi et al., 1998; Calavas et al., 1998), with an increased incidence of clinical mastitis being observed in the early lactation until weaning (Mork et al., 2007; Arsenault et al., 2008; Gougoulis et al., 2008). In the case of subclinical mastitis, a prevalence ranging from 5% to 30% has been found (Bergonier and Berthelot, 2003; Contreras et al., 2003; Berthelot et al., 2006). Bulk milk SCC (bSCC) has been used as an efficient and cost effective tool, in order to determine the epidemiology of subclinical mastitis at flock level (Lagriffoul et al. 1999; Bergonier et al., 2003).

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Isolation Rate (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurellaceae</td>
<td><em>Mannheimia</em></td>
<td><em>M. haemolytica</em>, <em>M. glucosids</em>, <em>M. muntzialis</em></td>
<td>0.5-2.0</td>
<td>Mavrogianni et al. (2007), Omaleki et al. (2010)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td><em>Escherichia</em></td>
<td><em>E. coli</em>, <em>Klebsiella</em></td>
<td>0.5-6.5</td>
<td>Fthenakis et al. (2004), Mork et al. (2007), Neihels et al. (2008)</td>
</tr>
<tr>
<td>Mycoplasmataceae</td>
<td><em>Mycoplasma</em></td>
<td><em>M. agalactiae</em>, <em>M. orpinosuniae</em>, <em>M. conjunctivae</em></td>
<td>0.0-60.0</td>
<td>Ostrovnikov and Jankowskii (2014)</td>
</tr>
<tr>
<td>Streptococcaceae</td>
<td><em>Streptococcus</em></td>
<td><em>S. agalactiae</em>, <em>Streptococcus spp.</em></td>
<td>0.0-75.0</td>
<td>Zhang et al. (2005), Contreras and Rodriguez (2011), Spanu et al. (2011)</td>
</tr>
<tr>
<td>Corynebacteriaceae</td>
<td><em>Corynebacterium</em></td>
<td><em>Corynebacterium spp.</em></td>
<td>3.5-11.0</td>
<td>Leitner and Krifucks (2007), Contreras and Rodriguez (2011)</td>
</tr>
<tr>
<td>Pseudomonadaceae</td>
<td><em>Pseudomonas</em></td>
<td><em>P. aeruginosa</em></td>
<td>0.0-20.0</td>
<td>Leitner and Krifucks (2007), Contreras and Rodriguez (2011)</td>
</tr>
<tr>
<td>Bacillaceae</td>
<td><em>Bacillus</em></td>
<td><em>Bacillus spp.</em></td>
<td>3.0-6.5</td>
<td>Arsenault et al. (2008), Spanu et al. (2011)</td>
</tr>
<tr>
<td>Burkholderiaceae</td>
<td><em>Burkholderia</em></td>
<td><em>B. cepacia</em></td>
<td>5.0-17.0</td>
<td>Berriatua et al. (2001)</td>
</tr>
<tr>
<td>Clostridiaceae</td>
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<td><em>C. perfringens</em></td>
<td>2.0-15.0</td>
<td>Mork et al. (2007), Forou et al. (2011)</td>
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<tr>
<td>Enterococcaceae</td>
<td><em>Enterococcus</em></td>
<td><em>E. durans</em>, <em>E. faecalis</em>, <em>E. faecium</em></td>
<td>0.5-10.0</td>
<td>Marogna et al. (2010)</td>
</tr>
<tr>
<td>Mycobacteriaceae</td>
<td><em>Mycobacterium</em></td>
<td><em>M. avium</em></td>
<td>0.0-45.0</td>
<td>Neblia et al. (2009)</td>
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<tr>
<td>Nocardiaceae</td>
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<td><em>N. fuscina</em></td>
<td>0.0-15.0</td>
<td>Maldona et al. (2004)</td>
</tr>
<tr>
<td>Listeriaceae</td>
<td><em>Listeria</em></td>
<td><em>L. monocytogenes</em></td>
<td>0.0-5.0</td>
<td>Winter et al. (2009), Brugère-Picoux (2008)</td>
</tr>
</tbody>
</table>
RISK FACTORS

Flock management

Housing
Incorrect housing conditions may lead to clinical and subclinical mastitis. Substandard shed hygiene due to inappropriate stocking density, straw bedding and ventilation, increases the incidence risk of intramammary infections (Caroprese, 2008). Additionally, insufficient disinfection programs can promote the multiplication of environmental pathogens, which increases the risk of mastitis (Bergonier et al., 2003). In any case, housing conditions are strongly related to the overall management scheme, with permanently housed, intensively reared ewes, being more exposed to environmental pathogens in the sheep shed, when compared to semi-intensively or semi-extensively reared ewes (Gelasakis et al., 2010).

Feeding
Improper feeding may lead to clinical and subclinical mastitis. According to Koutsoumpas et al. (2013), lack of vitamin A may predispose to both clinical and subclinical mastitis. Similarly, vitamin E deficiency during the dry period, as well as increased gossypol consumption have been reported to contribute to occurrence of clinical mastitis (Fthenakis et al., 2004; Giadinis et al., 2011).

Preventive medicine-health problems
Among preventive medicine practices, the use of intramammary infusions of antibiotics, during dry period when implemented inappropriately, may facilitate the colonization of the teat duct canal with pathogens (bacteria, fungi), resulting in ascending intramammary infections (Las Heras et al., 2000; Bergonier and Berthelot, 2003). In any case, inadequate health status of the ewe plays a crucial role towards mastitis manifestation, affecting both animals’ overall hygiene condition and their immune system function (Sordillo, 2005). Based on the results of recent studies by Mavrogianni et al., (2012, 2014) it can be assumed that trematodes and nematodes infections during dry period and lactation, respectively, may increase the incidence of mastitis. Also, teat lesion due to Orf virus infection forms a significant predisposing factor of intramammary infections resulted from local immunity relaxation (Mavrogianni et al., 2007).

Physiological factors
According to Waage and Vatn (2008), prolificacy may have a significant impact on the incidence of mastitis in meat ewes. Similarly, in dairy ewes, increased incidence risk of intramammary infections has been reported for ewes suckling two or more lambs (Arsenaault et al., 2008; Koop et al., 2010); this could be due to teat lesions caused by teat bites during the frequent suckling events of lambs (Fragkou et al., 2007).

The stage of lactation is associated with the occurrence of intramammary infections. For example, Bergonier et al. (2003) indicated higher incidence of clinical mastitis, caused by S. aureus, at the
first weeks of lactation. Also, Kirk and Glenn (1996) and Leitner et al. (2001) observed a high prevalence of subclinical mastitis at the early stages of lactation. On the contrary, new intramammary infections during drying-off period are rarely observed, with the exception of sporadic cases (Las Heras et al., 2000, Leitner et al., 2001).

A relationship between the number of lactation and the sanitary status of the mammary gland has been documented by Leitner et al. (2001), who found a significantly positive correlation between parity number and intramammary infections incidence. On the contrary, Sevi et al. (2000) reported that mastitis infections were set in progressively earlier as parity decreased. A possible explanation could be the fact that natural defense mechanisms are less efficient in younger ewes.

Genetic factors

In dairy sheep breeds, the correlations between udder morphological traits, milkability and SCC have been found to be significant, indicating a relationship between udder conformation and its hygiene (Marie et al., 1999). According to Casu et al. (2010), Sarda×Lacaune backcross ewes with deep and pendulous udder and high implanted teats are more susceptible to overmilking which predisposes to intramammary infections. In another study, Gelasakis et al. (2012) reported that in Chios breed ewes, the combination of small size and horizontally placed teats coupled with inappropriate udder conformation can predispose to mastitis. Poor machine milkability results either in overmilking or retained milk in the udder cistern with negative effects on the mammary glands’ sanitary status (Gelasakis et al., 2012).

The last decade, there has been an increased interest in studying the genetic basis of mastitis. In France, genetic analyses for mastitis resistance reported genetic predisposition to mastitis (Barillet et al., 2001; Rupp et al., 2006; Barillet, 2007). Recently, Bramis et al. (2014) investigated the genetic predisposition of mastitis in purebred Chios dairy ewes in Greece, using genomic analyses with a customized 960 SNP DNA array earlier developed as a part of the 3SR Project funded by the European Commission (Rupp et al., 2013; Sechi et al., 2013). Several polymorphisms were identified that affected mastitis related traits, as described in the next chapter.

CONTROL OF MASTITIS

Controlling mastitis is a challenging task for dairy sheep flocks, both in terms of sustainable production and for maintaining high health and welfare standards. A holistic approach should be considered for the most effective control of the possible risk factors, which are underlying intramammary infections. Within this approach the parameters needed to be implemented are listed below.

Treatment of mastitis during lactation

There are two operating axes for the effective treatment of mastitis (Mavrogianni et al., 2011); a) the immediate initiation of therapy and b) the intramammary antibiotherapy combined with subcutaneous or intravenous administration, in cases where generalized signs coexist. There are a large number of formulations which contain a wide spectrum or combination of narrow range antibiotics. Given that very few of them have special approval for dairy ewes, products with approval for cattle can be used as extra label use, taking into consideration the peculiarities on the withdraw period needed to be kept, for the milk produced for human consumption. Nowadays, beta-lactamines and macrolides are widely used under field conditions (Bergonier et al., 2003). Also, Bergonier and Berthelot, (2003) mentioned the necessity of complementary treatment by the parenteral administration of anti-inflammatory drugs. In addition, Kiossis et al. (2007) reported a program which led to limitation of subclinical mastitis during lactation using a 3-day intramuscular treatment with Penethamate hydroiodide in affected animals. In the same study, the results showed that the health status of udders entering the dry period was better.

General husbandry measures

In order to avoid intramammary infections due to environmental pathogens, general husbandry measures and high hygiene standards must be applied. Appropriate stocking density (2.0 to 2.5 m2 depending on the floor type) bedding material (the quantity is determined by the type of material used) and ventilation (47 to 66 m3/hour) are crucial factors for the determination of the hygiene status inside the barn.
cient use of milking machine by the staff; this further requires the training of the staff on standard milking and general operating procedures. Following procedures similar to the ones applied by dairy cattle farmers, with species-oriented adaptations, warrants effective and hygienic milking, but, peculiarities of the farm (i.e. flock size), the staff and the animals should be always taken into consideration (Bergonier and Berthelot, 2003). For dairy ewes, Gonzalo and Marco, (1999) proposed the following operating parameters for the milking machine: 36 kPa vacuum level, 180 pulsations per min and 50% pulsation ratio.

For the cleaning of pipes and clusters the use of drinking water is suggested. In any other case, the bacteriological status of the water used should be periodically determined, in order to avoid intramammary infection outbreaks (Las Heras et al., 1999, Bergonier et al., 2003).

Prevention of new intramammary infections can be facilitated by using post-milking teat dipping. Several studies have revealed the effectiveness of the method, particularly, in highly infected flocks (Paape et al., 2001; Bergonier and Berthelot, 2003; Contreras et al., 2003). Different disinfectants have been proposed for teat disinfection with chlorexidine, dodecyl benzene sulfonic acid, glycerol monolaurate, hydrogen peroxide and iodophors being some of the most commonly used (Nickerson, 2001). As resistance of bacteria against some of the aforementioned disinfectants has been reported, special attention needs to be paid on the appropriate implementation of teat dipping and the effectiveness of the disinfectant itself.

Nutrition

Oral administration of vitamins A and E and selenium during the last month of gestation and lactation has a positive impact both on performance and on immunological status of ewes and their lambs (Rock et al., 2001; Rooke et al., 2004; Koutsoumpas et al., 2013). In general, appropriate feeding and balanced nutrition are significant for the assurance of a high health status of the mammary gland.

Drying-off treatment

The aim of drying-off treatment is to eliminate
bacteriological agents of infected mammary glands and prevent new infections at the beginning of the subsequent lactation period (Gonzalo et al., 2004; Schwimmer et al., 2008). Thus, drying-off treatment has been found to significantly reduce the incidence of clinical mastitis in dairy ewes (Gonzalo et al., 2004; McDougall and Anniss, 2005). According to Fthenakis et al. (2012), intramammary administration of antibiotics at the beginning of drying-off period should be part of health management programs during pregnancy. In such programs there is a debate whether generalized dry-off antibiotic therapy is preferable than selective treatment (Bergonier et al., 2003) as a spontaneous cure rate at parturition can be high for dairy ewes (about 20–60%) (Paape et al., 2001; Contreras et al., 2003; Bergonier and Berthelot, 2003). At the end of lactation, the lack of SCC threshold, as a selection criterion for ewes with subclinical mastitis, renders the selective intramammary treatment rather difficult to be established at flock level. This temporarily supports the generalized approach, in which case the overall cure rate of treated ewes may range from 65 to 95% (Ahmad et al., 1992; Chaffer et al., 2003).

Vaccination

Although there is no evidence that vaccines available on market are effective, they are widely used in cases of high incidence of gangrenous mastitis (Bergonier and Berthelot, 2003; Bergonier et al., 2003). Only a few studies have reported a remarkable effectiveness of vaccination against mastitis caused by S. aureus in dairy ewes (Amorena et al., 1994, Tøllersrud et al., 2002). According to Melero (1994), a reduced prevalence of clinical mastitis but not of subclinical infections was observed, when a vaccine against S. aureus and S. Simulans was used. In some cases the use of autovaccines may be proposed, with unspecified results, though. Based on these observations, vaccination against mastitis related pathogens does not seem to be an effective tool for the prevention of intramammary infections in dairy ewes.

Genetic resistance

Breeding for resistance to mastitis in dairy cattle has been the subject of research over the last decades (Herringstad et al., 2003; Odegard et al., 2005; Morris, 2007). The most sustainable method for mastitis control via genetic resistance is selective breeding; it requires a suitable selection trait or a molecular genetic marker, with adequate additive genetic variation of this trait (Chang et al., 2004; Odegard et al., 2005). Because of the high and positive genetic correlation between SCC and mastitis, selection for reduced SCC can be used as an indirect selection trait for the control of mastitis. Nowadays, there is significant evidence that there are adverse genetic correlations between resistance to mastitis and milk production traits in dairy cattle (Philipsson and Lindhe, 2003), but not in dairy sheep, yet. For this reason, 12 countries have included bovine mastitis in their breeding programs in order to control the increased genetic susceptibility to it resulted from the selection for increased milk yield (Mark et al., 2002).

A few years ago, Barillet et al. (2001) carried out for first time a genetic analysis for mastitis resistance in the French Lacaune breed. The goal of this study was to define a breeding strategy for improved udder health in dairy sheep using SCC as an indirect trait. In other studies, it was revealed a strong negative genetic relationship between SCC and milk yield during the first lactation (Rupp et al., 2006; Barillet, 2007). Moreover, researchers have found a genetic relationship between udder morphology traits and udder health in dairy sheep (Legarra and Ugarte, 2005; Sechi et al., 2007; Casu et al., 2010). In Greece, Gelasakis et al. (2012) reported that improvement of milkability, in Chios dairy ewes, can be achieved by genetic selection programs using udder traits associated with udder morphology, teat size and placement which is expected to further improve the sanitary status of the mammary gland. Significant differences regarding mastitis susceptibility, among different sheep breeds have, also, been observed. For example, Fragkou et al. (2007) reported a higher resistance against Mannheimia haemolytica for Karagouniko comparing with Frizarta ewes, due to a lack of lymphoid nodules in Frizarta ewes’ teats. Although, these results need to be taken into consideration for genetic selection against mastitis, further analyses are necessary to validate their repeatability at population level in order to be used for breeding schemes.
in specific breeds.

The detection of the ovine genome regions involved in mastitis resistance began with the first results of a Quantitative Trait Loci (QTL) detection program (Barillet, 2007). According to the results, QTLs for SCC were detected on chromosomes 6 and 16, allowing locating the genes that control resistance to mastitis. Swiderek et al. (2006) described a possible correlation between Toll-like receptor (TLR)-gene mutations and the susceptibility of the mammary gland to bacterial infections. This study also showed the associate breed-dependent aspects of SCC, bacterial infection and TLR-gene mutations in sheep and its data may serve as a benchmark for further study of TLR-gene mutation, facilitating the identification of one of the markers of natural resistance against sheep mastitis.

Within a European Commission funded research project (“3SR”), putative polymorphisms for ovine mastitis resistance have been found for Lacaune, Sarda and Churra sheep breeds (Rupp et al., 2013; Sechi et al., 2013). Based on these polymorphisms a 960 Single Nucleotide Polymorphisms DNA array was used for the validation of the effects of the selected markers on the mastitis resistance for Chios dairy ewes (Psifidi et al., 2014). These markers were found to be suitable for genetic selection against mastitis programs without compromising productivity potential of Chios dairy ewes (Psifidi et al., 2014). As a result of the same project, recently, Bramis et al. (2014) found that other than SCC mastitis-related traits (total viable counts and CMT score) were heritable and thus suggested that they could be used as indirect selection traits when selecting for mastitis resistance in Chios dairy ewes.

CONCLUDING REMARKS

Bacterial mastitis is an important and highly multivariable disease of dairy sheep presenting varying degrees of clinical manifestation and consequences. The characteristics of the intramammary infections leading to mastitis in conjunction with breeding and management particularities of dairy sheep emerge the need to establish specific mastitis control programs. Hence, a holistic approach including appropriate management practices (housing, feeding and nutrition, milking routine, preventive health) and targeted genetic selection should be considered for the most effective control of intramammary infections and mastitis. In particular, selective breeding for resistance against mastitis should be assessed as a tool for the most effective control of mastitis. Towards this target, several aspects need to be taken into consideration and further investigations are needed in order to reveal possible negative effects of selecting against mastitis on productive potential of dairy ewes.

CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

REFERENCES


