



# Mastitis in sheep – The last 10 years and the future of research



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## ABSTRACT

Bacterial mastitis is a significant welfare and financial problem in sheep flocks. This paper reviews the recently published literature, including publications that highlight the significance and virulence factors of the causal agents, especially *Staphylococcus aureus* and *Mannheimia haemolytica*, the primary causes of the disease. Research has also contributed to the understanding of risk factors, including genetic susceptibility of animals to infections, supporting future strategies for sustainable disease control. Pathogenetic mechanisms, including the role of the local defenses in the teat, have also been described and can assist formulation of strategies that induce local immune responses in the teat of ewes. Further to well-established diagnostic techniques, i.e., bacteriological tests and somatic cell counting, advanced methodologies, e.g., proteomics technologies, will likely contribute to more rapid and accurate diagnostics, in turn enhancing mastitis control efforts.

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## 1. Introduction

Mammary infections can lead to various clinical or subclinical diseases in sheep. These are bacterial mastitis ('mastitis'), mycoplasma mastitis (contagious agalactia) and lentiviral mammary infection. The objective of the present review is to discuss bacterial mastitis, as contagious agalactia (Corrales et al., 2007; Gomez-Martin et al., 2013) and lentiviral infections (Minguijón et al., 2015) have been recently reviewed. The term 'mammary infection' includes only microbial diseases of the mammary parenchyma, hence infections of the teats and the udder skin, e.g., *Papilloma virus* infection of teats, contagious ecthyma (orf), impetigo (staphylococcal dermatitis) or sheep pox, are not discussed in the present review.

Mammary infections are the primary causes of 'Milk-drop syndrome in ewes' (>85% of all causes) (Giadinis et al., 2012). The syndrome has been defined as a pathological entity at flock level, characterised by reduced milk yield of lactating ewes, with no clinical signs specific to a disease (Giadinis et al., 2012).

In dairy-type flocks, mammary infections have an obvious financial significance, due to the reduction in milk yield, the downgrading of milk quality and the rejection of milk after antibiotic administration. Nevertheless, mammary infections are important also in meat production flocks, as reduced milk yield of ewes has been shown to lead to suboptimal growth of their lambs (Fthenakis and Jones, 1990a). Other costs associated with the

disease include those for replacement ewes and the relevant veterinary expenses.

In sheep, mammary infections are also of great welfare concern (European Food Safety Authority, 2009). Clinical mastitis is a disease that leads to anxiety, restlessness, changes in feeding behavior and pain in affected ewes (Fthenakis and Jones, 1990b). Even in subclinical mastitis, normal behavioral patterns of sheep are modified (Gougoulis et al., 2008a, 2010), hence raising potential welfare concerns.

In recent years, there has been an increased interest, internationally, in the study of ovine mastitis, with increasing number of doctoral theses published at various universities around Europe. Moreover, the European Commission has awarded a research grant ('3SR–Sustainable solutions for small ruminants') to an international consortium, which aimed to identify and promote means of sustainable control of the disorder; some of the findings of that project will be reviewed in this paper. Finally, the conclusions of a recent meeting of a working group in 'Welfare of sheep' of the Animal Health and Welfare Panel of the European Food Safety Authority, of which one of the authors (GCF) was an invited expert member, indicated that mastitis is one of the three most important problems adversely affecting welfare of sheep across the range of sheep production and management systems (European Food Safety Authority, 2014).

Several review papers have discussed earlier research in ovine mastitis (Watson and Buswell, 1984; Menzies and Ramanoo, 2001; Bergonier et al., 2003; Contreras et al., 2007). This paper focuses in research findings that have been published in the last 10 years and discusses potential opportunities for future research.

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## 2. Methodology

The review includes primarily references published in journals cited at the Web of Knowledge database ([wok.mimas.ac.uk](http://wok.mimas.ac.uk)); papers published in these journals have been refereed. Various search terms have been employed to identify relevant publications (e.g., 'sheep', 'goat\*', 'mastitis', 'somatic cell count\*', 'milk', 'Staphylococcus', 'Mannheimia', 'teat'). Subsequently, the full papers have been retrieved through the websites of the respective journals.

Moreover, selected papers from the proceedings of scientific meetings, mainly held in 2012 to 2014, are also discussed. Those present a means for quick publication of recent, relevant research in the field and the contents of proceedings of these meetings have been edited before publication.

## 3. Aetiological agents

Several bacterial agents as detailed below, have been found to be associated with clinical or subclinical mastitis in ewes (Contreras et al., 2007). Most often, these have been identified based in the use of conventional phenotypic identification systems. Differences have been identified in the aetiological agents of the disease related to the production system. In dairy production systems, coagulase-negative staphylococci or *Staphylococcus aureus* are the principal causes of subclinical or clinical mastitis, respectively (Bergonier et al., 2003; Mørk et al., 2005; Contreras et al., 2007). In meat production systems, most cases of clinical mastitis are associated with *Mannheimia haemolytica* or *S. aureus* (Mavroggianni et al., 2007; Arsenault et al., 2008; Koop et al., 2010; Omaleki et al., 2010). An overview of recent studies regarding incidence and etiology of ovine mastitis is presented in Table 1.

### 3.1. *Staphylococcus aureus*

*S. aureus* is the most common mastitis-related pathogen in sheep (Mørk et al., 2005; Mavroggianni et al., 2011). Bergonier et al. (2003) indicated that *S. aureus* was the major mastitis-causing agent, isolated from sporadic cases or outbreaks of the disease. The organism is responsible for about 40% of cases in ewes suckling lambs and 80% of cases in milking ewes (Mørk et al., 2007; Koop et al., 2010; Mavroggianni et al., 2011).

*S. aureus* strains are divided in four *agr* (accessory gene regulator) groups (I, II, III, IV), according to the *agr* locus, which regulates production of virulence factors (Novick et al., 1995; Vautor et al., 2007). Based on this classification, rapid discrimination of *S. aureus* strains has been proposed (Goerke et al., 2005). Vautor et al. (2007) have used this classification to study the distribution of *S. aureus* strains from milk of ewes with mastitis and from nostrils of sheep, into the four *agr* groups; they found that most isolates could be assigned to groups I (44%) or III (46%).

In recent years, antibiotic resistant *S. aureus* strains have been recognised as an emerging threat for public health. In several studies, the antibiotic resistance patterns of different *S. aureus* isolates from cases of ovine mastitis have been described (Fthenakis, 1998; Goñi et al., 2004; Mørk et al., 2005; Vautor et al., 2007); moreover, the potential of resistant strains of the organisms in dairy products from sheep milk and the potential consequences for public health have been discussed (Normanno et al., 2007). Lollai et al. (2008) evaluated antibiotic susceptibility of 1284 strains of *S. aureus* from ovine mastitis cases and found increased resistance rate against streptomycin (48–87%), with smaller resistance rates found against penicillin or ampicillin (2–12% or 0–12%, respectively). Other studies have nevertheless grouped staphylococcal strains from mastitis independently of coagulase-production type and have reported increased resistance

to penicillin G (up to 31%) or to ampicillin (up to 30%) (Corrente et al., 2003; Kunz et al., 2011; Onni et al., 2011).

### 3.2. Other staphylococci

Coagulase-negative staphylococci are pathogens of smaller virulence, associated mostly with subclinical intramammary infections (Bonnefont et al., 2011), although they may also cause clinical disease (Fthenakis and Jones, 1990b). *Staphylococcus epidermidis* is the most common species associated with ovine mastitis (Onni et al., 2010), followed by *Staphylococcus chromogenes*, *Staphylococcus simulans* and *Staphylococcus xylosum*; less prevalent species include *Staphylococcus auricularis*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus cohnii*, *Staphylococcus equorum*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus lentus*, *Staphylococcus muscae*, *Staphylococcus saprophyticus*, *Staphylococcus sciuri* and *Staphylococcus warneri*. Table 2 summarises the coagulase-negative staphylococcal species reported in various studies in the last 10 years. The general findings are in accord with results of earlier studies (Fthenakis, 1994; Bergonier et al., 2003) regarding the role of coagulase-negative staphylococci in subclinical ovine mastitis.

Recently, it has been recognised that antimicrobial susceptibility of coagulase-negative staphylococci, which represent the majority of organisms isolated from ovine milk, is important for the early recognition of newly emerging resistant milk-borne bacterial agents (Onni et al., 2011).

Additionally, *Staphylococcus hyicus*, *Staphylococcus intermedius* and *Staphylococcus schleiferi* (all coagulase-positive species) have been isolated, with a low frequency, from cases of clinical mastitis in sheep.

### 3.3. *Mannheimia spp.*

*M. haemolytica*, *M. glucosida* and *M. ruminalis* have been isolated from cases of acute mastitis in sheep (Omaleki et al., 2010, 2011), with *M. haemolytica* confirmed as the most common cause of mastitis in flocks in meat type producing systems (Arsenault et al., 2008; Koop et al., 2010; Omaleki et al., 2010). In contrast, in dairy sheep, only 11% of cases of intramammary infections were found to be caused by *M. haemolytica* (Mavroggianni et al., 2007).

### 3.4. *Streptococcus spp.*

*Streptococcus spp.* are sporadic pathogens of ovine mastitis (Zdragas et al., 2005; Contreras and Rodríguez, 2011). Increased incidence of mastitis caused by these organisms is usually associated with inappropriate housing conditions or milking practices, as found in studies of the disease in Italian or German flocks, where incidence risks of 23–31% have been reported (Marogna et al., 2010; Cuccuru et al., 2011; Kern et al., 2013).

### 3.5. Other bacteria

Other Gram-positive bacteria associated with intramammary infections include *Bacillus cereus* (usually, after intramammary administration of antibiotic tubes performed under suboptimal conditions of hygiene), *Clostridium spp.* (Mørk et al., 2007; Fotou et al., 2011), *Corynebacterium spp.* (Spanu et al., 2011), *Enterococcus spp.* (*Enterococcus faecalis*, *E. faecium*, *E. durans*; Marogna et al., 2010), *Listeria monocytogenes* (Winter et al., 2004; Brugère-Picoux, 2008), *Micrococcus spp.* (Ariznabarreta et al., 2002), *Mycobacterium spp.* (including *Mycobacterium avium* subsp. *paratuberculosis*; Nebbia et al., 2006) and *Trueperella pyogenes* (Saratsis et al., 1998; Hadimli et al., 2010).

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