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REVIEW ARTICLE

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Predisposing factors for bacterial mastitis in ewes

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Abstract

Objective of this review paper is the appraisal of predisposing factors for bacterial mastitis in ewes. Factors that predispose ewes to mastitis can be classified into non-animal-related factors (environmental and climatological factors, housing, nutrition, milking practices) and animal-related factors (anatomic, genetic, litter size—ethological factors, number and stage of lactation period, health problems). There are clear management—environment—animal interactions in mastitis development in ewes, which underline its multifactorial nature. Research and studies regarding risk factors are important, in order to develop strategies for their elimination, control or correction. Control measures for bacterial mastitis, which attempt to eliminate predisposing factors for infection, can thus be developed and implemented.

KEYWORDS

ewe, mastitis, predisposing factor, risk factor, sheep

1 | INTRODUCTION

In sheep, bacterial mastitis may be caused by a variety of micro-organisms. The main aetiological agents of clinical mastitis are *Staphylococcus aureus* in dairy ewes and *S. aureus* and *Mannheimia haemolytica* in non-dairy ewes. Coagulase-negative staphylococci (e.g. *Staphylococcus epidermidis*, *Staphylococcus simulans*) are the primary causal agents of subclinical mastitis. Various other organisms (e.g. *Escherichia coli*, streptococci etc.) account for a smaller proportion of cases of the disease (Bergonier & Berthelot, 2003; Bergonier, Cremoux, Rupp, Lagriffoul, & Berthelot, 2003; Gelasakis, Mavrogianni, Petridis, Vasileiou, & Fthenakis, 2015; Queiroga, 2017).

The adverse financial effects of mastitis are summarized below:

- Death of affected ewes.
- Loss of affected mammary glands and consequent early culling of ewes.
- Replacement costs, due to early culling of affected ewes.
- Veterinary expenses.
- Reduced milk production by affected ewes.
- Downgrading of the quality of milk produced, due to changes in its composition.
- Discard of milk unsuitable for human consumption, due to increased content of bacteria, bacterial toxins or drug residues.
- Increased mortality and reduced growth rate of lambs of affected ewes.

Moreover, in ewes, mastitis is of significant welfare concern (European Food Safety Authority, 2014). Clinical mastitis is a disease that leads to anxiety, restlessness, changes in feeding behaviour and pain (Fthenakis & Jones, 1990b; Gougoulis, Kyriazakis, & Fthenakis, 2010; Gougoulis, Kyriazakis, Papaioannou, et al., 2008a).

Various factors predispose animals to the disease and increase risk of its incidence. Research in predisposing factors of ovine mastitis can contribute in the elucidation of disease mechanisms and thus in the development and implementation of control measures, which take into account conditions on sheep farms. The objective of this review paper is the appraisal of predisposing factors for mastitis in ewes. A basic classification scheme of predisposing factors includes animal-related factors and non-animal-related factors (Table 1).

2 | NON-ANIMAL-RELATED PREDISPOSING FACTORS

2.1 | Climatological factors

The potential effects of climatological factors in mastitis development were first indicated by Clark (1972), who mentioned that

mastitis was occurring more frequently after gusts of cold winds or grazing in wet and muddy pastures, in rainy weather. A possible association between adverse weather conditions and mastitis in sheep has also been reported in the 1980s (Anon, 1988). More recently, Vasileiou et al. (2019) have presented an association between increased mastitis frequency and the mean temperature for 30 days prior to sampling date (positive correlation) and the minimum temperature of coldest month (negative correlation). Based on those findings, it has become possible to construct predictive models for mastitis in ewes taking into account climatological parameters (Giannakopoulos et al., 2019).

Sevi et al. (2001) have reported that the provision of shade helped minimizing the impact of thermal stress in the immune function and udder health of ewes. High environmental temperatures can lead to reduced leucocyte counts in sheep (El-Tarabany, El-Tarabany, & Atta, 2017). Also, in such cases, leucocytes have impaired function (e.g. Lacetera et al., 2005; Lecchi, Rota, Vitali, Ceciliani, & Lacetera, 2016). Leucocytes play an important role in mammary defences; therefore, any reduction in their numbers and efficacy may impede the defensive ability of ewes and contribute to an increased incidence of mastitis in flocks, where extreme temperatures prevail. Furthermore, during high environmental temperatures, ewes have a reduced feed intake, which can lead to limited availability of energy, thus adversely affecting mammary defences (Barbagianni et al., 2015).

Low temperatures can also lead to mastitis. In cows, it has been reported that colonization of teat skin by staphylococci was more intense in such conditions (Fox & Norell, 1994; Zucali et al., 2011). Intramammary infections during cold weather can be a consequence of increased incidence of chapped teats (Fox & Hancock, 1989). Burriel (1997) has reported that colder and wetter weather prolonged survival of *M. haemolytica* in the environment of sheep, which in turn could increase cumulative bacterial counts, contributing to easier transmission from animal to animal. The increased susceptibility can result from physicochemical changes in chapped teat skin (Mavrogianni, Cripps, Papaioannou, Taitzoglou, & Fthenakis, 2006). In such conditions, there is decreased concentration of lipids (including antibacterial fatty acids), bacteriostatic salts, proteins (e.g. teat duct keratin) and immunoglobulins in the epidermis (Noble & Somerville, 1974). Furthermore, chapped skin is dehydrated, which results in changes in its microflora, leading to decreased resistance to bacterial colonization (Fox & Cumming, 1995). Additionally, chapping causes removal of the acid mantle, which reduces protection, and results in excoriation and fissuring, increasing the total surface area of the teat and enabling increased opportunities for bacteria to attach (Sieber & Farnsworth, 1984). During periods with reduced environmental temperatures, increased incidence of teat chapping (Fox & Hancock, 1989) and sucking frequency by lambs increases (Slee & Springbett, 1986) have been reported; concurrent occurrence of these two factors increases bacterial transfer onto the teat skin, increasing the risk of colonization and entry of bacteria into the teat duct, leading on to the development of mastitis (Fragkou et al., 2011; Gougoulis, Kyriazakis, Tzora, et al., 2008b).

With regard to precipitation, Zafalon, Santana, Pilon, and Júnior (2016) have reported higher frequency of subclinical mastitis in ewes during rainiest periods. This is probably due to difficulties in maintaining environmental hygiene.

Despite environmental and climatological factors being to a large extent outside of the control of farm managers, these findings can be valuable in establishing principles of udder health management in sheep flocks. Hence, in areas found to have increased risk for mastitis development (Giannakopoulos et al., 2019), relevant preventive measures for mastitis need to be established and applied appropriately. Moreover, during periods of environmental conditions leading to high risk for mastitis development, preventive measures should be applied meticulously to minimize risk of mammary infections.

TABLE 1 Classification of factors that may predispose ewes to mastitis

Non-animal-related factors	Animal-related factors
Climatological factors	Anatomic factors
Housing and general hygiene in farms	Genetic background
Nutrition	Litter size—ethological factors
Milking practices	Number and stage of lactation period
	Health problems

2.2 | Housing and general hygiene in farms

Poor housing practices (e.g. overstocking, limited ventilation, irregular manure removal) can contribute to increased bacterial numbers within animal barns, thus increasing the risk of mastitis development; for example, Caroprese (2008) has reported that when <7 m^3 per sheep were available within animal houses, there was increased risk of mastitis. Indrebø (1991) has studied specific sheep housing features related to mastitis prevalence and reported higher prevalence rates in sheep houses with slatted floors. However, Cooper, Huntley, Crump, Lovatt, and Green (2016) have reported that concrete and soil were associated with higher incidence rates of clinical mastitis.

Frequent manure removal from farm buildings has been associated with reduced bacterial counts in milk (Sevi, Albenzio, Muscio, Casamissima, & Centoducati, 2003).

Dirt and faeces on teat skin can increase risk of mastitis by environmental pathogens (Jones, 1990). Moreover, several insects, for example the fly *Hydrotoea irritans*, may transfer bacteria from teats of ewes with mastitis, which have higher bacterial numbers, to teats of healthy ewes (Jensen & Swift, 1982; Jones, 1990).

2.3 | Nutrition

Balanced nutrition of lactating ewes supports mammary health by improving the immunological competence of the animals (Caroprese, Giannenas, & Fthenakis, 2015). Nutritional errors may, through

various pathways, affect the immune abilities of the ewes and predispose the animals to mastitis (Waage & Vatn, 2008).

With regard to energy, it has been reported that pregnancy toxaemia has a marked effect on the immune functions of affected ewes and can predispose ewes to mastitis in the immediately post-partum period (Barbagianni et al., 2015). Increased ketone body blood concentrations have been found to lead to reduced blastogenesis and proliferation of T lymphocytes (Franklin, Young, & Nonnecke, 1991), whilst lymphocytes from ketonaemic animals showed reduced mitogenic responses than lymphocytes from healthy cows (Kandefer-Szerszen, Filar, Szuster-Ciesielska, & Rzeski, 1992). Sartorelli, Paltrinieri, and Agnes (1999) have shown that increased concentrations of β-hydroxybutyrate could adversely affect particle uptake by neutrophils; Hoeben, Heyneman, and Burvenich (1997) reported that increased concentrations of β-hydroxybutyrate led to an inhibitory effect on the respiratory burst activity of neutrophils, by inhibiting the generation of superoxide anions; Grinberg, Elazar, Rosenshine, and Shpigel (2008) have shown that increased concentrations of β-hydroxybutyrate suppress formation of extracellular traps by neutrophils, which leads to impaired pathogen killing. All above pathways participate in impairment of various mammary defence mechanisms.

Vitamin A deficiency has been found to lead to increased incidence of mastitis and increased milk somatic cell counts (Koutsoumpas et al., 2013). This may be due to an impairment of mammary epithelium resulting from the deficiency, which supports invasion of pathogens into the mammary parenchyma and multiplication therein.

Giadinis et al. (2011) have also indicated a possible predisposing role of selenium deficiency in mastitis. Reduced selenium concentration may lead to a deficient cellular defence, which in turn can result in a higher incidence of clinical mastitis. Selenium-containing proteins, mainly GSH-P_x and thioredoxin reductase, are involved in the metabolism of cellular peroxides; therefore, selenium deficiency impairs the ability of immune cells to produce the respiratory burst (Morgante et al., 1999; Rooke, Robinson, & Arthur, 2004).

Zinc deficiency can adversely affect keratin formation in the teat; this would lead to limited protection of ewes from bacterial invasion and increase the potential for mastitis development. Saianda, Bettencourt, Queiroga, Ferreira-Dias, and Vilela (2007) have indicated that the administration of a zinc proteinate feed supplement to ewes resulted in reduced bacterial adhesion onto mammary epithelial cells, possibly due to maintaining epithelial integrity in treated ewes.

Fthenakis, Leontides, Skoufos, Taitzoglou, and Tzora (2004) presented a case of >90% prevalence of staphylococcal mastitis in ewes provided with a feed with excessive (20%) gossypol content. The authors postulated that the increased consumption of gossypol had resulted, through various pathways, in the affected ewes becoming immunocompromised, predisposing them to mastitis (Fthenakis et al., 2004).

It is generally believed that the presence of aflatoxins in feed might lead to higher incidence of mastitis. However, Kourousekos (2011), who reviewed the relevant literature, discussed that a likelihood of potential aflatoxin effects on udder health could not be supported by the available evidence.

2.4 | Milking practices

Various practices during the milking routine, for example wrong milking order of ewes, incorrect teat preparation (e.g. incomplete dirt removal) or stressing animals (impairing oxytocin release and complete emptying of mammary glands), can predispose to mastitis. Also, the practice of providing feed during milking may contribute; after milking and feeding, ewes would lay on the ground, thus facilitating bacterial entrance through the open teat orifice and leading in mammary infections.

In machine-milked ewes, various inappropriate practices can increase the risk for mammary infection, due to bacterial entrance into the mammary gland and/or compromise of teat defence mechanisms. Bacterial entrance is associated with factors like reverse milk flow, impacts or reverse pressure gradient (Billon, Sauvee, Menard, & Gaudin, 1998; Rasmussen, Frimer, & Decker, 1994); this can be further increased in cases of milking machine malfunction, which may affect teat status, causing lesions predisposing to mammary infection (Fragkou, Papaioannou, Cripps, Boscos, & Fthenakis, 2007a). Moreover, various errors in milking system management, which include application of clusters on wet and slippery teats, use of damaged clusters, incorrect vacuum level or pulsation rate of the system, errors in post-milking cleaning of the system (e.g. suboptimal temperature of cleaning water, omission of detergents) or leaks in air or milk pipes, would significantly increase risk for mastitis development in ewes. Application of automated cluster removal has been found to limit vacuum drops in short milk tubes and oedema at teat end after milking (Bueso-Ródenas, Romero, Arias, Rodríguez, & Díaz, 2015) and may thus contribute in maintaining mammary health.

In hand-milked ewes, staphylococci from the hands of milkers can be transmitted to the teats of ewes (Marco Melero, 1994). Likely as a consequence of that, hand-milking has been found to result in increased bacterial colonization of the teat duct (Mavrogianni, Cripps, & Fthenakis, 2007). More recently, Vasileiou, Chatzopoulos, et al. (2018a) have reported that hand-milking was a risk factor for mastitis caused by biofilm-forming staphylococci; possibly, milkers contribute to transmission of these bacteria to sheep (Vasileiou, Chatzopoulos, et al., 2018a).

The potential effects of post-milking teat dipping in ewes have not been studied as extensively as in cows (Bergonier et al., 2003; Vasileiou, Cripps, et al., 2018b). Nevertheless, omission of the practice can lead to a 3-fold increase in the prevalence of subclinical mastitis (Vasileiou, Cripps, et al., 2018b). Incorrect application of teat dipping can also predispose to mastitis: use of dense solutions may cause teat chapping, which increases risk of mammary infection (Fragkou, Papaioannou, et al., 2007a; Mavrogianni et al., 2006), whilst contaminated products may result in mastitis by sporadic pathogens (e.g. *Serratia marcescens*) (Tzora & Fthenakis, 1998).

Finally, in contrast to traditional views, there is now evidence that the drying-off process (i.e. abrupt or progressive) does not influence incidence risk of mastitis. Hence, it should not be regarded as a risk factor for mastitis development (Petridis et al., 2012, 2013).

3 | ANIMAL-RELATED PREDISPOSING FACTORS

3.1 | Anatomic factors

Various factors, which modify the normal morphology and standard conformation of the udder, can predispose ewes to mastitis. For example, udders with irregular conformation and large pendulous udders ("baggy udders") would predispose to mastitis, perhaps because the tips of the teats may be closer to the ground and thus are more likely to come into contact with environmental bacteria (Fthenakis, 1992; Larsgard & Vaabenoe, 1993), whilst ewes with firm udders have been found to produce milk with lower somatic cell counts (Huntley, Cooper, Bradley, & Green, 2012). The teat length and diameter can also play a role in the infection of mammary glands; long teats are considered to contribute in minimizing mammary infections, as, being longer, there is possibly more time available for the defence mechanisms of the teat (Mavrogianni et al., 2005) to better counteract bacterial infections. In wider teats, infection can take place more easily and are considered to contribute to increased risk of mastitis. Bad conformation of teats (e.g. misplaced teats, small-sized teats) can predispose ewes to mastitis, particularly in flocks in which mechanical milking is applied. Such teats often lead to poor milkability of ewes, due to difficulties in application of milking machine clusters, as teats do not fit properly and thus are subjected to inappropriate pressure and manipulation during milking, increasing the risk of bacterial infection (Fthenakis, 1992). Finally, supernumerary teats predispose to mastitis, as they provide more chances for infections of the mammary glands (Fthenakis, 1992).

3.2 | Genetic background

Differences in breed susceptibility to mastitis have been identified. This has been documented in ewes of high milk production. For example, the Frisarta breed, a high milk yield breed, has been found to be susceptible to clinical mastitis after bacterial deposition into the teat, whilst Karagouniko breed ewes have been found to be resilient to inoculation (Fragkou, Skoufos, et al., 2007b). Also, Vasileiou, Gougoulis, et al. (2018c) have found that the prevalence of subclinical mastitis in high-yielding breeds was significantly higher than the national average prevalence recorded during a nationwide investigation in Greece. Similar theories have been developed regarding meat-producing flocks in New Zealand by Ridler (2008).

The various anatomical characteristics that may contribute to increased or decreased incidence of mastitis (Section 3.1) often have a genetic background, hence, through their heritability genetic factors influence mastitis development (Legarra & Ugarte, 2005). Also, Barillet, Rupp, Mignon-Grasteau, Astruc, and Jacquin (2001), McLaren et al. (2018) and Oget, Tosser-Klopp, and Rupp (2019) have mentioned that cell content in milk can be employed for selection of animals potentially resistant to mastitis, whilst Riggio, Portolano, Bovenhuis, and Bishop (2010) have proposed the use of infection status (defined as the presence of at least 5 colony-forming units per 10 μL of milk) for this purpose. By using these criteria, differences in outcomes have been found in lines of sheep considered to be resistant or susceptible to mastitis. Barillet et al. (2001) have concluded that selection for mastitis resistance based on somatic cell counts was feasible, although results obtained during the first lactation period of evaluated ewes should not be taken into account in relevant analyses.

Candidate genes implicated in innate immunity and thus resistance to mastitis included *SOCS2*, *CTLA4*, *C6*, *C7*, *C9*, *PTGER4*, *DAB2*, *CARD6*, *OSMR*, *PLXNC1*, *IDH1*, *ICOS*, *FYB* and *LYFR* in Chios breed (Banos et al., 2017). Fragkou et al. (2010) have attributed the breed differences in susceptibility of mastitis to differences in lymphocyte activity and proposed a potential genetic background to the infection, given that in cattle, the genetics of bovine leucocyte antigen (*BoLA*) has been linked to resistance to mastitis (Takeshima & Aida, 2006).

It is interesting that in the past, Traoré et al. (2008) have hinted at a possible link between resistance to mastitis and gastrointestinal nematodes. More recently, experimental and field studies have provided evidence of an association between gastrointestinal parasitic infections and mastitis in ewes (Section 3.5).

3.3 | Litter size—Ethological factors

Increased litter size has been positively associated with the development of mastitis (Prpic, Vnucec, Benic, & Mioc, 2016; Waage & Vatn, 2008). As there is no genetic association between number of ovulations and mastitis susceptibility (Oget et al., 2019), a reason for this should be considered the higher chance for bacterial transfer from lambs mouths to the teat duct (Fragkou et al., 2011; Gougoulis, Kyriazakis, Tzora, et al., 2008b). Moreover, ewes bearing multiple foetuses have increased energy needs during the last stage of gestation (Russell, 1984), which can predispose to pregnancy toxaemia and mastitis immediately post-partum (Barbagianni et al., 2015).

In a detailed study, using molecular techniques, it has been found that identical *M. haemolytica* isolates were recovered from the tonsils of lambs and the teat duct of their dams, results that proved the transmission of bacteria during sucking. It has been found that during mastitis, sucking patterns of offspring of affected ewes were modified and became irregular, whilst cross-sucking with other ewes was also recorded (Gougoulis, Kyriazakis, Papaioannou, et al., 2008a). This indicates that lambs may also transmit bacteria to ewes other than their dams.

3.4 | Number and stage of lactation period

There is evidence that increased age and number of lactation period have been associated with higher incidence of mastitis (Ahmad,

Timms, Morrical, & Brackelsberg, 1992; Fthenakis, 1994; Leitner et al., 2001; Sevi, Taibi, Albenzio, Muscio, & Annicchiarico, 2000; Watkins, Burriel, & Jones, 1991). More specifically, the probability of the mammary gland remaining uninfected is reduced as the stages of a lactation period advance (Mavrogianni et al., 2007). Fragkou et al. (2010) have postulated that this might be the effect of lymphocytic mammary defences becoming defective with age (Kraft et al., 1987).

In ewes, frequency of clinical mastitis immediately post-partum is not as high as in cows (Bergonier & Berthelot, 2003). At that stage of the lactation period, *Escherichia coli* predominates as a causal agent (Jones & Lanyon, 1987; Vasileiou, Cripps, et al., 2018b). Clinical mastitis can occur more often when lambs are taken away from their dams and milking starts (Bergonier & Berthelot, 2003; Fthenakis & Jones, 1990a). At that stage and thereafter, staphylococci are the primary causal agents of the infection (Vasileiou, Cripps, et al., 2018b).

3.5 | Health problems

Parasitic (nematodes, trematodes) infections may predispose to increased incidence risk of mastitis. Proposed pathogenetic mechanisms include the energy depletion by helminths, affecting leucocytic function in affected animals, thus impeding efficient mammary defences (Kordalis et al., 2019; Mavrogianni et al., 2017). Moreover, specifically in trematode infections, increased β-hydroxybutyrate blood concentrations, consequently to the liver damage caused by the parasites, can have a direct effect in function of leucocytes (Mavrogianni et al., 2014).

Contagious ecthyma (orf) and *papillomavirus* infections in teats have a significant predisposing role in mastitis. These epitheliotropic viruses hamper relevant local defences (specifically, the lymphoid nodules at the border between teat duct and teat cistern) (Mavrogianni et al., 2006) and thus facilitate bacterial multiplication at the teat and invasion into the mammary parenchyma. Other teat disorders (e.g. chapped teats) (Fragkou, Papaioannou, et al., 2007a) or staphylococcal dermatitis of udder skin (impetigo) may also predispose to mastitis; in these cases, increased risk for infection is related to high bacterial accumulation in udder and teat skin.

Pregnancy toxaemia can also predispose to mastitis in the immediately post-partum period of the subsequent lactation period (section 2.3). Dystocia has also been associated with mastitis in the subsequent lactation period (Waage & Vatn, 2008); in this case, the association may be based on the same background as pregnancy toxaemia, given that the disorder often results in dystocia (Barbagianni et al., 2015).

4 | CONCLUDING REMARKS

The reviewed works have studied and presented findings related to many factors that can predispose ewes to mastitis. These works have increased available knowledge regarding mastitis in sheep, and their value in understanding the disease is invaluable. Nevertheless, many of these have discussed the significance of the various factors in isolation, that is without taking into account other factors that may also influence development of mastitis. In this paper, the various factors and their significance for predisposing to mastitis are classified and presented in detail for the first time, with regard to their involvement in predisposing to mastitis. In practice however, interactions between all these factors also occur.

In mastitis, there are clear management—environment—animal interactions; hence, the significance of the above factors should be considered within that frame. In general, mastitis is considered to be more frequent in dairy flocks; however, that may only reflect the frequent udder monitoring in such farms, rather than indicating a true difference between dairy and non-dairy flocks. In a recent study performed in Greece, that had evaluated the importance of various factors and their interactions by using multivariable analysis, it emerged that only the management system applied in farms (European Food Safety Authority, 2014) was of importance for mastitis (Vasileiou, Cripps, et al., 2018b). The results indicated that mastitis occurred more frequently in farms managed under an intensive or semi-intensive system (Vasileiou, Cripps, et al., 2018b). "Management system" includes various aspects of flock husbandry and organization (e.g. housing facilities, nutrition, milking routine, animal breed); many of these have been described to affect development of mastitis in ewes. Also, "management system" encompasses many factors, which can contribute to subclinical mastitis or its control.

Thus, it becomes evident that mastitis is a multifactorial disease, with many factors potentially predisposing its development. Hence, its control also requires many approaches at various levels. Studies into risk factors shed light in the development of the disease and can help to achieve their elimination or correction. This approach supports sustainable prevention of the disease, as it leads to the reduced use of antimicrobial agents for the treatment of disease cases. Moreover, control measures for mastitis, which attempt to eliminate possible predisposing factors, can thus be developed and implemented.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

The review has been written as part of the PhD thesis of the first author; other authors are academic supervisors.

DATA AVAILABILITY

Data sharing not applicable—no new data generated.

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