Mastitis

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Inflammation of the mammary gland (mastitis) is an important disease of dairy sheep. Mastitis management depends mainly on the diagnosis. Conventional diagnostic methods including somatic cell count, California Mastitis Test, and microbial culture have limitations. Therefore researchers are looking for new diagnostic biomarkers of mastitis including specific proteins produced by the liver in case of disease (acute phase proteins), unique genetic sequences (miRNAs), or antimicrobial peptides produced by immune cells during inflammation (cathelicidines).

Keywords: ewe ; udder inflammation ; ovine ; milk quality

1. Introduction

Mammary gland inflammation (mastitis) is one of the most costly and severe diseases in the dairy industry [1]. Negative impact does not only refer to economic reasons but also significantly contributes to animals' health and consequently their welfare. Another important perspective is the food safety (food-borne diseases) and quality of dairy products (such as cheese) since milk from affected animals may contain pathogenic bacteria and has altered composition undesired by the dairy industry [2][3]. From a global point of view, the most important dairy species are cattle, producing over 80% of world milk production [4] followed by buffaloes with 15%, goats with 2% (accounts for about 1000 million head, of which 20% intended for dairy production) and sheep with 1% (accounts for almost 1200 million head, of which 25% intended for dairy production); camels provide 0.5% and 1.5% comes from other dairy species [4]. Consequently, mastitis in cattle is a well-recognized problem, to which researchers and bovine practitioners all around the world pay special attention. Nevertheless, in some countries due to climate and/or traditional or historical reasons milk is derived from other than cattle dairy species, for example, from small ruminants [5]. Goats and sheep are often kept in an environment with scarce grazing and unfavorable climatic conditions. In some countries, they are considered as dairy animals of the poor because of the lower capital investment and low production costs required [6]. They are also characterized by rapid generation turnover (and thus earlier milk production compared with other dairy animals), short pregnancies, and milk supply in quantities that are suitable for immediate household consumption (thereby reducing problems of milk storage and marketing) [5].

However, at the same time, small ruminants become more and more popular in highly developed countries since they perfectly fit into the conception of organic farming or are kept as pets, in particular in suburban areas [7][8]. Although sheep and goats are not demanding animals, they can provide products of great quality. Ewe's milk contains higher levels of total solids (protein and fat) and more major nutrients than goat and cow milk. To compare, sheep milk contains 5.5 ± 1.1 g/100 g of protein and 5.9 ± 0.3 g/100 g of fat, while cow milk contains 3.4 ± 0.1 g/100 g and 3.3 ± 0.2 g/100 g of these solids, respectively [9]. Consequently, ewe's milk is characterized by excellent cheese-making properties, thus is consumed rarely in liquid form. Apart from that, there is one compound in ruminants' milk—conjugated linoleic acid (CLA), the most abundant in sheep milk, that may have far-reaching, positive effects on milk consumption [10]. CLA has been shown to have numerous potential benefits for human health, including potent cancer-fighting properties [10]. Sheep milk-producing farms represent a significant part of the agrarian economies in many countries, especially those bordering the Mediterranean Sea and in the Middle East [9]. Moreover, it is important to highlight that dairy sheep are suitable for organic agriculture, involving a long period of grazing, great care for animal welfare, and reduced use of antibiotics and hormones. The bio (organic) products derived from organic farming are more and more popular in highly developed countries and this trend is expected to continue [11].

Mastitis in Dairy Sheep

Sheep are believed to be one of the first domesticated species and probably the problem of udder inflammation has been present since then [1]. Regarding mastitis in sheep the literature reports individual milk yield losses of 2.6–43.1% [2], being modulated by several factors including infection severity, production level, causal agents, and unilateral or bilateral infection. Mastitis not only negatively affects milk yield but also alters milk quality [7]. The impairment of physical and chemical characteristics due to decreased udder health status is responsible for the negative effect of increased somatic cell count on the coagulation properties of milk, the curd yield, and the quality of cheese [2], which does not allow producers to meet the quality standards required by consumers, industry and public health organizations [12]. A low ratio of casein to protein in high bulk tank somatic cell count (BTSCC) milk enhances the extension of the rennet coagulation time and curd firming time because there are more serum proteins, and the stability of casein micelles are reduced as a result of hydrolysis. Those changes, in turn, led to poor syneresis, lower cheese yield, increased moisture content, and lower fat and protein content in cheese [2].

Another important issue is public health in terms of consuming cheese made from infected milk, in particular, some traditional kinds of cheeses without milk pasteurization. Globally only 25% of sheep are intended for dairy production [13]. In many countries, most sheep are kept for the production of meat and therefore most studies focus on symptoms of mastitis occurring in ewes that are nursing lambs. In these flocks, only severe clinical mastitis is likely to be observed and diagnosed. According to Ruegg [14], this lack of emphasis on milking ewes has led to an over-emphasis on the occurrence of clinical mastitis and a lack of appreciation for subclinical mastitis. Clinical mastitis (CM) typically occurs in <5% of lactating ewes, but subclinical mastitis (SM) may occur in 15–30% of animals [14]. The information regarding mastitis prevalence in different management systems is given in **Table 1** . Among the etiologic agents, the most prevalent are Coagulase-negative staphylococci (CNS), Corynebacterium sp., while Streptococcus spp. , Enterobacteriaceae , Pseudomonas aeruginosa , Mannheimia haemolytica , Corynebacterium spp. , and fungi can also cause mastitis in sheep, but are observed at relatively lower rates [1]. Mastitis is considered one of the most significant reasons for premature culling in dairy sheep in the United Kingdom [15]. In the United States, udder-health issues account for about 14% of ewes culled each year [16]. There is no general consensus about the prevalence of mastitis in sheep of different breeds and from various areas. It has been reported that the culling of ewes resulting from clinical mastitis episodes can reach up to 70% [17] or even 90% [1]. Therefore, proper diagnosis is a critical aspect of preventing mastitis and its economic consequences. A significant role in mastitis control in the flock seems to have the udder health management at the end of the lactation period when the mammary gland is particularly prone to infections [18] and immediate or delayed culling. The elimination of existing subclinical infections relies on the intramammary application of antibiotics at drying-off and removing ewes affected by acute or chronic mastitis from the flock until culling or complete recovery [19]. Nevertheless, to identify the ewes with SM, laboratory diagnostics is crucial.

Table 1. Prevalence of subclinical ovine mastitis in different management systems.

Management System	Prevalence of Subclinical Mastitis	References
Semi-intensive	**0.296**	[20]
	0.120	[21]
	0.112	[22]
Intensive	**0.254**	[20]
Semi-extensive	**0.196**	[20]
	0.192	[23]
	0.139	[24]
Extensive	**0.178**	[20]
	0.192	[23]

2. Conventional Approach to Mastitis Diagnosis and Promising Novel Inflammatory Markers

2.1. Conventional Approach to Mastitis Diagnosis

Laboratory diagnostics play an important role in the improvement of production efficiency and the control of diseases including mastitis [24]. Mastitis cases might be classified as clinical or subclinical. The clinical form is characterized by abnormalities in milk (i.e., presence of blood, pus, color change, or lumps), palpable possible mammary gland tissue alteration, or systemic symptoms (e.g., fever, loss of appetite) [3]. On the other hand, the subclinical form involves the mobilization of inflammatory cells to the udder, increasing the somatic cell count (SCC), but without alterations in gland tissue or milk aspect, thus are hard to identify under field conditions [25]. One of the on-the-spot tests such as the California Mastitis Test (CMT) can help to diagnose SM in ewes, but unlike in dairy cattle, the CMT is infrequently used to detect increases in bulk milk somatic cell counts, although it may have some application in dairy sheep. CMT has some limitations because it was originally developed for cows, and also due to its subjective nature, which can lead to inaccuracies in the interpretation of the results [24][25].

Likewise, there is no consensus in the literature regarding a cut-off value of SCC in sheep milk [24][26]. Moreover, even if the SCC in milk is considered a standard indicator of SM in cows it may not be a specific sign of the inflammatory status of the mammary gland in sheep, due to variability due to numerous factors other than intramammary infections including age, breed, management system, physiological stage of the animal (lactation stage, dry period), season, numbers of lambs born, and other factors [27]. Individual SCC are not commonly used in sheep to detect and treat subclinical mastitis.

Nevertheless, many authors have attempted to set a cut-off value for the somatic cell count in ewe's milk; however, the data are still not consistent. Albenzio et al. [26] suggested that SCC > 300,000 cells/mL results in decreased milk production by the mammary gland. Other authors reported that the udder is considered healthy when the number of somatic cells does not exceed 250,000 cells/mL [28][29][30]. While Świderek et al. [31] stated that fluctuations of somatic cells in ewe's milk up to 200,000 cells/mL are normal and only above this value are considered as the possible threshold for subclinical mastitis. In contrast, Miglio et al. [27] suggested that uncertain subclinical mastitis occurs when bacteriological testing is positive, or SCC > 500,000 cells/mL (non-specific-SM) in milk samples. Spanu et al. [32] presented that in ewes with 3 or more monthly SCC ≥ 400,000 cells/mL, detection of mastitis pathogens in their milk was 5.6–7.5 times more likely, compared to ewes with SCC below this threshold. According to Kern et al. [33], the limit of 400,000 cells/mL would be the most suitable one for detecting problems of mastitis in meat sheep breeds. On the other hand, Olechnowicz and Jaśkowski [1] suggested that in dairy sheep, SCC between 200,000 and 400,000 cells/mL indicates subclinical mastitis.

Another relevant diagnostic tool is microbiological culture, which aims to isolate the pathogen causing the infection. Consequently, the milk sample should be incubated in a culture medium and checked for colony growth. Further identification of bacteria is also required, which is time-consuming. This method has disadvantages; particularly the length of time it takes for testing as well as the frequent incidence of 'no growth' cases in mastitis milk cultures. Other diagnostic tools and markers of mastitis in dairy sheep include collecting samples from the udder tissue of infected ewes and subsequently performing histological and immunohistochemical analyses have been described, but concurrently impractical and implemented mostly in the research studies [34].

However, all these tests have their shortcomings that necessitate the introduction and development of more sensitive and reliable predictors of mastitis. An increase in SCC and positive bacteriology for mastitis pathogens in milk samples are indicative of subclinical mastitis but the evidence of only one of these alterations must suggest an uncertain case of subclinical mastitis [27]. Unfortunately, animals with SM often remain untreated because the disease may not be revealed and this creates a real thread for animal health, farm income, and public health. Nevertheless, SCC and bacteriological examination are expensive, time and labor-consuming, and are not yet in use at the farm level in dairy ewes. Therefore, researchers are looking for a new promising diagnostic tool as following the world-recognized mastitis expert, who claims that investments in defining mastitis control strategies for minor dairy species (such as dairy sheep, goats, and buffalo) are needed [35].

There are many reports in the literature confirming that genetics has a significant impact on mastitis control and diagnosis in ewes [36]. The effect of allelic polymorphism of different genes on SCC has been demonstrated. For example, Świderek et al. [37] established significant differences between SCC level and the percentage of CD4, CD8, and CD19 lymphocytes in the milk depending on the alleles of the Ovar-MHC genes (OLADRB1, OLADRB2, OMHC1). In sheep milk contained $<200 \times 10^3$/mL SC, they indicated 488 bp (DRB1) and 284 bp (DRB2) more frequently. However, in milk contained $>200 \times 10^3$/mL somatic cells, they detected 508 bp (DRB1) and 272 (DRB2) alleles more often. Additionally, Sutera et al. [38] identified few candidate genes associated with SCC in Valle del Belice sheep related to immunity system and udder conformation. They detected eight significant SNPs (single nucleotide polymorphisms) for SCC located in five different chromosomes, and among these, only one marker reached the genome-wide significance threshold. The most significant SNP associated with SCC is located in the region of $SERP_1$ (stress-associated endoplasmic reticulum protein 1). Moreover, these results suggest that individuals with the GG genotypes at rs401598547, CC at rs161717499, and AA at rs403091159, rs422960374, and rs426621433, could be selected to reduce the content of the somatic cell in milk [38]. On the other hand, research conducted by Banos et al. [39] confirmed the presence of animal genetic variability in mastitis resistance and identified genomic regions associated with specific mastitis traits in the Chios sheep. For this research, they genotyped 609 ewes with a custom-made 960-single nucleotide polymorphism DNA array based on markers located in quantitative trait loci (QTL) regions for mastitis resistance. SNP markers located in 5 chromosomes and relevant candidate genes implicated in innate immunity ($SOCS_2$, $CTLA_4$, C6, C7, C9, $PTGER_4$, DAB_2, CARD6, OSMR, $PLXNC_1$, IDH_1, ICOS, FYB, and LYFR) were indicated [39].

All of these studies confirmed that searching for and learning about new genetic polymorphisms will facilitate the diagnosis and prevention of mastitis, and also will indicate new directions for breeding work including genetic selection. There is a need to consider genetic improvement for reduced susceptibility to mastitis, as a sustainable means to control the disease. However, selection for resistance to mastitis is difficult because of its polygenic nature, where many genes with small effects are involved [20].

Promising Novel Inflammatory Markers of Mastitis in Sheep

A permanent search is underway for other indicators of inflammation that would enable more efficient, sensitive, and specific detection of mastitis. These indicators should constitute an alternative to SCC or as a supplement for evaluation or improving SCC performance [40][41]. Researchers should look for the molecules that are released into the milk by the mammary gland during inflammation. This indicator should be a molecule, enzyme, or protein that is practical for detection with enzymatic assays or other immunoassay procedures [40]. Recent investigations and data carried over from humans, as well as veterinary medicine, show that acute-phase proteins (APP), microRNAs (miRNAs; short, non-coding RNAs), and cathelicidins measurement may be the tools needed to improve the early diagnostics of ovine mastitis [42][43][44].

3. Summary

Mastitis is a complex and severe disease in ewes causing significant losses to the dairy industry. It is important to note that its management depends mainly on diagnostics. Therefore, novel biomarkers with higher sensitivity and accuracy are highly desired in this matter. These include acute-phase proteins, miRNAs, and/or cathelicidins. Currently, these markers are under intensive research, but in the future, they could potentially be applied in routine veterinary diagnostics.

It is important to highlight that application of reviewed biomarkers is currently limited. Up to date the combination of bacteriological and cytological examinations is considered to be the most reliable means of diagnosing ovine subclinical mastitis. Although, they are not as widely used as in dairy cattle. The limitations of reviewed potential novel biomarkers may have different causes. On the one hand, the cost of laboratory analyses seems to be relatively high (e.g., specialized equipment is needed for proteomic or miRNAs analyses). In particular, taking into account the sheep and its milk economic value. On the other hand, the proposed biomarkers in sheep are currently under intensive research and their role is not fully understood. Therefore, further studies are required to assess their utility as diagnostics tools in ovine mastitis. In the future probably, if the cost of proposed biomarkers laboratory analyzes would become lower, then they would gain significance.

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