An Overview of Specific Pathogens in Goat Mastitis

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Bulletin UASVM Veterinary Medicine 76(2)/2019

Print ISSN 1843-5270; Electronic ISSN 1843-5378

doi:10.15835/buasvmcn-vm:2019.0025

Abstract:

Goat milk ranks fourth in terms of global milk production and lately it has become increasingly popular among consumers. Unfortunately, mastitis is one of the most common diseases that affects dairy goats, with serious economic consequences and food safety matters. The prevalence of clinical mastitis is lower than 5% and the main aetiological agent is *S. aureus*. On the other hand, the prevalence of subclinical mastitis is between 5-30%, with coagulase negative staphylococci (CNS) representing the most often isolated microorganisms.

The aim of this paper is to highlight the main aspects regarding the aetiology of goat mastitis, as well as the importance of the milk somatic cell count (MSCC) in the diagnosis process. Although the inflammation of the mammary gland in goats is not as frequently diagnosed compared to cow mastitis, there are several aspects worth discussing in order to fully understand the pathogenesis of intramammary infections.

Keywords: aetiology, goat, mastitis, milk somatic cell count

Introduction

Mastitis is defined as the inflammation of the mammary gland. Most often, the inflammatory process occurs as a result of intramammary infections with different pathogenic agents, but also physical, chemical or mechanical injuries can lead to mastitis (Accorsi *et al.*, 2002). Regardless of the species, factors affecting the udder health are considered to be limiting factors in the development of the dairy industry, which lead to major economic consequences resulting from significantly decreased milk production and expensive veterinary services, especially antibiotic treatments (Ajuwape *et al.*, 2005).

Mastitis in small ruminants is considered to be highly important from several points of view. First of all, the economic aspect is the one

that prevails, the diseases of the mammary gland resulting in quantitative and qualitative decrease of milk yield, along with the prescription of costly therapeutic protocols. Furthermore, severely affected animals are, most often, slaughtered. It is also worth mentioning the importance of the hygiene and safety criteria of milk and dairy products, with the risk of illness occurring among consumers, due to the consumption of possibly contaminated products. Nevertheless, the legal aspects regarding the microbiological quality of the milk should be implemented and respected accordingly (Olechnowicz and Jaśkowski, 2014).

An important aspect regarding the pathology of mastitis is the concept of microbiota of the mammary gland. In recent years, the

sterility of healthy mammary tissue has been questioned, being disproved by the results of studies based on the presence of bacterial DNA. Thus, studies conducted on bovine milk concluded that there is an intramammary normal flora in the healthy udder composed of a variety of bacteria. Furthermore, it is stated that mastitis would be, predominantly, a consequence of the dysbiosis of the microbiota rather than a primary infection (Contreras and Rodriguez, 2011; Oikonomou *et al.*, 2012; Rainard, 2017).

To confirm this hypothesis, it has been shown that the microbial flora in milk samples from healthy cows is different from that of cows diagnosed with mastitis. Also, the presence of several bacterial species such as *Staphylococcus aureus* and *Streptococcus uberis* in healthy bovine milk samples, with a low number of somatic cells, reinforced the statement according to which bacteria that is normally found on the surface of the skin or intestinal mucosa are also a part of the commensal flora of the mammary gland (Oikonomou *et al.*, 2014).

Epidemiological aspects of goat mastitis

From a clinical point of view, mastitis is divided in two categories: clinical and subclinical mastitis. Clinical mastitis is defined as the inflammation of the mammary gland in which visible, local and even general symptoms occur, along with significant changes in milk secretion, while subclinical mastitis develops without clinical signs. In this particular case, the diagnosis protocol is based on milk examination. Additionally, clinical mastitis can be divided into three categories according to its evolution: peracute, acute and chronic mastitis. Peracute mastitis has a very rapid evolution and is most often gangrenous or serous. On the other hand, acute mastitis is divided into catarrhal and hemorrhagic mastitis, whilst chronic mastitis is often purulent (Groza *et al.*, 2006).

In small ruminants, the incidence of clinical mastitis is generally less than 5%, whereas subclinical disorders are considered to have an incidence ranging from 5 to 30% (Bergonier and Berthelot, 2003; Contreras *et al.*, 2003). Among the pathogens that may cause mastitis in sheep and goats, bacteria of the genus *Staphylococcus* are most commonly isolated, but other agents causing mastitis, such as *Streptococcus* spp., *Enterobacteriaceae*, *Pseudomonas aeruginosa*,

Mannheimia haemolytica, *Corynebacterium* spp. can be isolated, with a lower prevalence. Cases of mastitis produced by *Aspergillus fumigatus*, *Serratia marcescens* or *Burkholderia cepacia* are also reported (Kalogridou-Vassiliadou, 1990; Bergonier and Berthelot, 2003; Contreras *et al.*, 2003). Although *Streptococcus* spp. and Gram-negative bacilli are known to be major intramammary pathogens in cows, they are not very frequently isolated from mammary gland infections in the goat and are more likely associated with poor hygiene and milking conditions. In addition to the before mentioned pathogens, other microorganisms that can cause mastitis in goats, but with a lower frequency, are *Arcanobacterium pyogenes*, *Bacillus* spp., *Clostridium perfringens*, *Nocardia asteroides* (Contreras *et al.*, 2003).

Furthermore, *Mycoplasma* spp. represents another pathogenic agent that causes mastitis in small ruminants, being responsible for the evolution of the contagious agalactia syndrome. This disease has a high prevalence in the Mediterranean region, but it has also been reported in other parts of the world (Contreras *et al.*, 2003). Because various symptoms other than mastitis are observed in this disease, different authors do not consider *Mycoplasma* spp. as an aetiological agent of intramammary infections. However, the evolution of this syndrome results in reduced milk production and significant increase in MSCC. Due to this fact, some authors consider this pathogen as being one of the most important causes of mastitis in endemic areas, where the incidence of subclinical mastitis is high. In herds where contagious agalactia evolves, large economic losses are reported due to decreased milk production, mortality or slaughter of affected animals (Corrales *et al.*, 2004).

Regarding the evolution of clinical mastitis, *Staphylococcus aureus* is the main aetiological agent, with the highest prevalence, followed by coagulase-negative staphylococci (CNS), *Streptococcus* spp., *Enterobacteriaceae*, *Arcanobacterium pyogenes*, *Corynebacterium* spp., *Pasteurellaceae* etc. Enzootic or epizootic episodes are mainly due to the presence of *S. aureus*, *Streptococcus uberis*, *S. agalactiae*, *S. suis* or opportunistic pathogens, such as *Aspergillus fumigatus*, *Pseudomonas aeruginosa* and less commonly *Serratia marcescens* or *Burkholderia cepacia* (Las Heras *et al.*, 2000; Berriatua *et al.*, 2001; Bergonier *et al.*, 2003).

The persistence of clinical intramammary infections during lactation depends on the size of the herd and technological factors. If left untreated, animals in the acute phase become chronic, with the disease spreading for several months or longer. In the selected goat herds, 18% of the slaughtered animals undergo mastitis episodes. In both goats and sheep, mammary pathology is one of the main causes of culling, being more frequent in the first 2-3 months of lactation (Malher *et al.*, 2001). According to the study conducted by Sanchez *et al.* (1999), the incidence of clinical mastitis does not vary with the stage of lactation to the same extent as in cattle. A higher rate of mastitis is observed at the beginning of the milking period and in the first third of lactation.

As mentioned above, subclinical mastitis in goats is most commonly caused by CNS species, with a high prevalence of *Staphylococcus caprae* and *Staphylococcus epidermidis*, the latter being associated with the highest SCC, both in sheep and goats. The persistence of subclinical mastitis during lactation varies depending on the pathogen that has caused the infection, but it is generally high. The conditions that evolve subclinically are hardly detectable (Albenzio *et al.*, 2002; Bergonier *et al.*, 2003). In goats, a higher frequency regarding subclinical mastitis is observed at the beginning of lactation, the prevalence increasing along with the parity (Sanchez *et al.*, 1999).

The primary sources of infection are the carrier animals, but also the evolution of non-clinically detectable mastitis. Bacterial persistence in the environment is mainly due to technological errors regarding milking machines and poor hygiene. Furthermore, the high density of flocks and overpopulated shelters cause a decrease in the air quality and an increase in the concentration of the total number of germs, this particular aspect being associated with incorrect ventilation and increased humidity in the shelter (Bergonier *et al.*, 2003).

The main aetiological agents of caprine mastitis

a. *Staphylococcus* spp.

The genus *Staphylococcus* includes different species of ubiquitary present microorganisms, found on the epithelial surfaces and skin, both in humans and animals. They are resistant to drought, osmotic shock and temperature variations. When

the balance of the commensal microbial flora is disturbed and the defense mechanisms of the body are overturned, staphylococci multiply, causing local or even general infections (Andrei and Groza, 2010). Morphologically speaking, these microorganisms are round-shaped (cocci) that stain strongly gram-positive and form grape-like clusters. The number of species is constantly increasing, over 40 species and more than 20 subspecies being described, the main differentiating factor being the production of coagulase. Coagulase is an enzyme responsible for prothrombin activation, promoting plasma coagulation. Thus, *Staphylococcus* species are divided into coagulase-negative staphylococci (CNS), which do not produce coagulase and coagulase-positive staphylococci (CPS), this aspect being an indicator of pathogenicity (McVey *et al.*, 2013; Becker *et al.*, 2014).

By far the most frequently isolated pathogen from cases of goat mastitis is *Staphylococcus* spp. (Bergonier *et al.*, 2003; Marogna *et al.*, 2012; Rola *et al.*, 2015; Pirzada *et al.*, 2016). Intramammary infections caused by these pathogens are of particular importance, as these microorganisms are responsible for both the onset of clinical (gangrenous) and subclinical mastitis. *Staphylococcus aureus* is a CPS, characterised by a very high pathogenicity, very frequently involved in the aetiology of mastitis. It is shown that between 4 and 18% of bacterial intramammary infections are caused by this pathogen (Bergonier *et al.*, 2003; Contreras *et al.*, 2007; Dore *et al.*, 2016).

In terms of pathogenicity, *S. aureus* produces a series of toxins responsible for causing food poisoning among the human population consuming contaminated milk, even pasteurized, due to the presence of thermostable enterotoxins (Contreras *et al.*, 2007). Moreover, besides these enterotoxins, a number of virulence factors, such as leukotoxins, are also encountered.

These elements have the ability to destroy monocytes and polymorphonuclears (PMN) of the host. In a study conducted by Rainard *et al.*, 2003, it was demonstrated that the *S. aureus* strains isolated from goats and sheep with mastitis have a more pronounced leukotoxic activity on bovine polymorphonuclear cells than those isolated from large ruminants. The same study showed that PMN from small ruminants are more resistant to

the action of these toxins in comparison to PMN from cattle.

Another important issue is the isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) strains from goat mastitis. These strains are mostly responsible for nosocomial infections worldwide and their prevalence has increased considerably in recent years. The resistance to methicillin is mediated by the *mecA* gene, which encodes penicillin-binding protein 2a, making these strains resistant to methicillin or other β -lactam antibiotic. Such cases have been reported in investigations conducted by Stastkova *et al.* (2009), Aras *et al.* (2012), Caruso *et al.* (2016).

Coagulase-negative staphylococci (CNS) represent the category with the highest prevalence which causes mastitis in ruminants (Contreras *et al.*, 2003; Contreras and Rodriguez, 2011; Silanikove *et al.*, 2014; Gelasakis *et al.*, 2016). Although they are considered to be less pathogenic than *S. aureus*, CNS produce subclinical mastitis, with an increase of MSCC. The prevalence of SCN producing these disorders is 60% to 80% in goats and 45-48% in sheep (Olechnowicz and Jaśkowski, 2014; Persson *et al.*, 2015). The most commonly isolated SCN species from cases of subclinical mastitis in goats are: *Staphylococcus epidermidis*, *S. caprae*, *S. simulans*, *S. chromogenes*, *S. xylosus* (Bergonier *et al.*, 2003; Dore *et al.*, 2016).

Some authors have pointed out that in goats losses that occur due to subclinical infections are limited and that the SCC cannot be used to assess the severity of the infection. However, subclinical mastitis are known to significantly increase the MSCC (McDougall *et al.*, 2002). Intramammary infections with CNS do not have such a pronounced effect on SCC compared to *Staphylococcus aureus*, thus CNS can be considered minor pathogens in goats (Koop *et al.*, 2010; Koop *et al.*, 2012).

b. *Streptococcus* spp.

Streptococci are gram positive, catalase-negative cocci, that tend to form chains of variable lengths or pairs, being characterized by a pronounced ecological, serological and genetic diversity. They are ubiquitous, commensal germs of the upper respiratory and genital tract (McVey *et al.*, 2013). These microorganisms require optimal conditions for growth, with the addition of blood or serum in the culture medium. The pathogenic

strains are capsulated and form mucoid colonies (Quinn *et al.*, 2011).

Despite the fact that bacteria of the genus *Streptococcus* are of major importance regarding the pathology of mastitis in bovine, they do not have a high prevalence in goats. Therefore, streptococcal mastitis account for 5-10% of the total clinical mastitis cases reported in this species. In general, streptococci are responsible for the onset of clinical infections. In contrast to sheep, where *Streptococcus agalactiae* is the major pathogen involved in mastitis, studies conducted on goats have shown the absence of this species, showing that the poor hygiene is the main factor that leads to mastitis (Contreras *et al.*, 2003).

Streptococcus equi subsp. *zooepidemicus* has been isolated from mastitis cases in goats and sheep, as reported by Mallikeswaran and Padmanaban (1991) and Las Heras *et al.* (2000).

Streptococci produce virulence factors that include enzymes and exotoxins, such as streptolysins (hemolysins), hyaluronidase, streptokinases and proteases, but the polysaccharide capsule is the main virulence factor. Moreover, they produce a series of surface proteins, called adhesins, that bind to the host cell's extracellular matrix proteins, making the microorganisms resistant to phagocytosis. Generally speaking, pathogenic streptococci are susceptible to penicillins, cephalosporins, macrolides, chloramphenicol and sulfamides and are often resistant to aminoglycosides, fluoroquinolones and tetracyclines (McVey *et al.*, 2013).

c. Gram-negative bacteria

Escherichia coli is a gram-negative bacteria of the genus *Escherichia*, found in the digestive tract in both mammals and birds, producing local or systemic infections. From the large intestine, these bacteria are eliminated in the environment, thus polluting the soil, water, litter and shelters (Andrei and Groza, 2010). Morphologically speaking, *E. coli* is a rod-shaped bacterium, which possesses flagella, therefore is motile (McVey *et al.*, 2013).

Colibacillary mastitis in goats are less frequently diagnosed compared to those produced by gram-positive bacteria, this condition being characterized by the presence of both local and general symptoms. Thus, the affected mammary gland is edematous, congested and sensitive to palpation. These symptoms may also be

accompanied by digestive (diarrhea) and nervous disorders (paraplegia), fever or muscle tremors. At milking, the presence of a serous, yellowish pink secretion can be observed (Groza *et al.*, 2006).

The members of the genus *Pseudomonas* are involved in the aetiology of goat mastitis, but have a significantly lower prevalence compared to other bacterial species, being considered opportunistic agents. In general, mastitis caused by *Pseudomonas* spp. is due to poor milking hygiene, poor maintenance conditions and presence of contaminated water. In addition to this, these conditions may also be a consequence of inappropriate intramammary treatments. Such cases have been mainly reported in sheep flocks (Bergonier *et al.*, 2003; Contreras *et al.*, 2003). Although the genus *Pseudomonas* comprises more than 200 species and subspecies, most diseases in the veterinary sphere are caused by *Pseudomonas aeruginosa*. This species is of great importance in internal medicine, because most strains are resistant to commonly used antibiotics, so the infection is difficult to cure (Quinn *et al.*, 2011; McVey *et al.*, 2013).

d. *Mycoplasma* spp.

In goats, clinical mastitis caused by mycoplasmas has a high prevalence in some areas, causing much greater economic damage compared to mastitis produced by other pathogens. The contagious agalaxia of small ruminants is endemic in the Mediterranean basin, but is also reported in other regions of the world. The main consequence of the disease is the dramatic decrease in milk yield, which in some cases may be irreversible. Other clinical signs, such as keratoconjunctivitis, arthritis, pneumonia or abortion, may also occur (Contreras *et al.*, 2003).

These microorganisms are frequently found on the mucous membranes of the respiratory and urogenital tracts. In sheep, mastitis is mainly caused by *Mycoplasma agalactiae*, but in goats other species such as *M. mycoides* subsp. *capri*, *M. capricolum* subsp. *capricolum*, *M. putrefaciens* are frequently involved in the aetiology of mastitis. In addition to the etiological complexity underlying these infections, a major disadvantage regarding this disease is the poor efficiency of the control and prevention methods (Contreras *et al.*, 2003; Amores *et al.*, 2012; Kumar *et al.*, 2013).

Unlike other pathogens, mycoplasmas are not thought to produce specific toxins, but the production of hydrogen peroxide can induce toxic effects on the cells to the surface on which they adhere. Furthermore, some pathogenic species have certain surface proteins, which facilitate the attachment to cells. These proteins have a pronounced variability, which increases the pathogenicity of these microorganisms (Quinn *et al.*, 2011).

e. Other pathogens

In addition to the bacterial genera described above, which are frequently isolated from mastitis cases in goats, there are other microorganisms that can cause intramammary infections, but with a lower prevalence. Therefore, gram-positive microorganisms include bacteria of the genus *Arcanobacterium*, especially the species *Arcanobacterium pyogenes*, formerly known as *Actinomyces pyogenes* or *Corynebacterium pyogenes*. These bacteria produce, in most cases, clinical mastitis characterized by a significant decrease in milk production, which is sometimes irreversible (Contreras *et al.*, 2003).

Corynebacterium spp. represents another category of microorganisms that produce sub-clinical infections among goats, but are considered to be minor pathogens. Other microorganisms, such as *Nocardia* spp., *Bacillus* spp. and *Clostridium perfringens* have also been isolated from goat mastitis, but in sporadic cases (Kalogridou-Vassiliadou, 1991; Contreras *et al.*, 2003).

Aspects regarding diagnostic methods

The diagnosis of clinical mastitis is based on the examination of the mammary gland, which involves the inspection and palpation of breast tissue, milking, mammary biopsy or other complementary tests (ultrasound, endoscopy), as well as laboratory examinations of milk. On the other hand, subclinical mastitis is detected by milk testing. Thus, the following tests can be used: boiling milk test, pH determination, California Mastitis Test (CMT), reductase and catalase test, determining the electrical conductivity of milk and MSCC (Andrei and Groza, 2010).

The gold standard method used in the diagnosis of mastitis in ruminant species is bacterial culture. For economic, but also practical reasons, a single milk sample is used to diagnose the infection,

but in order to establish a correct diagnosis, it is necessary to isolate the microorganisms from samples collected consecutively, before and after milking. Due to the fact that the specificity of these tests has been shown to be higher for samples collected after milking, it is highly recommended that the latter should be used for diagnosis (Sanchez *et al.*, 2004; Contreras *et al.*, 2007).

The most important differences between goats and sheep regarding the diagnosis of mastitis are related to the MSCC. These differences relate mainly to the fact that MSCC is normally high in the healthy goats, due to the significant apocrine component of the mammary tissue, but also due to a large number of non-infectious factors leading to this increase in MSCC compared to the sheep. Therefore, in goats, several factors such as treatments, vaccinations, dietary modifications or stress have been found to increase MSCC (Paape *et al.*, 2001; Bergonier *et al.*, 2003). Because MCSS is an indicator of milk quality, it is important that its determination is as accurate as possible. Moreover, in goats, MCSS is not always a suitable indicator of mammary gland health, because a large number of cytoplasmic particles from the apical portion of the secretory cells are found in the milk of this species. Although most of these particles are anucleated, there is a possibility that some of them will contain fragments of the nucleus, leading to the increase of the cell count. The cytoplasmic particles are similar in size to the milk somatic cells, their concentration in the goat species being $150 \times 10^3/\text{ml}$ (Paape *et al.*, 2001). If the mammary gland health in cattle is confirmed by MSCC up to 100,000 cells/ml, in the goat that number can vary between 200,000 cells/ml and several million cells/ml (Stuhr and Aulrich, 2010). Furthermore, in goats, neutrophils represent the main cell type of milk somatic cells (approximately 80%), unlike cows or sheep, where macrophages are found in a greater percentage. Because neutrophils represent the first line of defense against infections, this fact could explain why goats are more resistant to mastitis than other animal species (Souza *et al.*, 2012).

Persson and Olofsson (2011) proposed a threshold of 345×10^3 cells mL^{-1} in order to differentiate between infected and healthy glands in goats, but the stage of lactation and parity should be taken into account when it comes to the interpretation of the MSCC.

It is well known that the increase in of MSCC is related to the pathogenicity of the agent that is involved in the aetiology of mastitis. Generally speaking, both the major and minor pathogens described above lead to an increase of MSCC, but in a study conducted by Bagnicka *et al.* (2011) bacterial pathogens have been isolated from milk samples with a low somatic cell count ($1 \times 10^6/\text{ml}$).

Another method used for detecting subclinical mastitis is measuring the electrical conductivity of milk. In case of an intramammary infection, changes in the permeability of mammary tissue occur, thus increasing the concentration of Na^+ and Cl^- in milk. This parameter is used to monitor the health of the mammary gland in cows, being less commonly used in small ruminants (Stuhr and Aulrich, 2010). Also, determining the enzymatic activity can be considered as an adjuvant in establishing a diagnosis. Thus, an enzyme present in large quantities during inflammatory processes is β -glucuronidase, with correlations being established between the concentration of this enzyme, the increased number of somatic cells and the presence of pathogens. According to Stuhr *et al.* (2013), β -glucuronidase activity in goats is not influenced by the stage of lactation, therefore this parameter could represent a better indicator for the detection of mastitis in dairy goats. In the same study mentioned above, non-infected halves showed much lower values in β -glucuronidase activity in comparison to bacteriologically positive milk samples.

Other milk enzymes that can be used are lactate dehydrogenase, alkaline phosphatase, aspartate-aminotransferase, but also catalase, lactoperoxidase and xanthine oxidase. Like glucuronidase, the concentration of lactoperoxidase in goat milk increases when MSCC also increases, which is also common in women and cattle (Stuhr and Aulrich, 2010; Stuhr *et al.*, 2013).

Conclusion

The inflammation of the mammary gland in goats is divided into clinical and subclinical disorders, with clinical mastitis having a lower prevalence, below 5%, while subclinical ones have a much higher prevalence. The most frequently isolated pathogen from cases of clinical mastitis is *Staphylococcus aureus*, and in the subclinical mastitis the highest prevalence is recorded by CNS, other agents being considered minor pathogens,

except for *Mycoplasma* spp. The latter causes significant economic damages in the regions where contagious agalactia syndrome evolves (Contreras *et al.*, 2003; Contreras *et al.*, 2007).

The diagnosis of clinical mastitis involves the examination of the mammary gland, whereas subclinical mastitis, without visible symptoms, requires complementary examinations of the milk secretion, in order to establish a correct diagnosis. Therefore, tests that are commonly used for cow milk can also be used for the goat milk, but with some limitations. The determination of the MSCC is not relevant, because the goats shed physiologically a greater number of such cells in the milk secretion, due to the significant apocrine component of the mammary gland and the numerous non-infectious factors that can increase this parameter. Therefore, several indicators should be taken into account when establishing a correct diagnosis for an intramammary infection in goats, as the MSCC does not stand alone for a positive diagnosis. The electrical conductivity, pH, and dosage of certain enzymes may be helpful in establishing a diagnosis (Stuhr *et al.*, 2013). However, the gold standard method for the diagnosis of bacterial mastitis remains the microbiological examination. Within the bacteriological exam, it is also recommended to test the susceptibility to antibiotics, in order to establish an adequate therapeutic protocol and avoid the antibiotic resistance phenomenon.

Acknowledgments. This research project was supported by research grant no. 37PFE/06.11.2018.

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