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Mastitis in small ruminants

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Abstract

Mastitis is a complex disease resulting from the interaction between the agent, animal, and environment, and is in most cases associated with the presence of microorganisms. It constitutes an important animal and public health problem, with worldwide economic repercussions. This literature review aimed to present the most recent studies on mastitis in goats and sheep, updating etiology, epidemiology, diagnosis, control, and prevention. Prevalence varies between 5 and 30% to subclinical mastitis, but staphylococcal mastitis is the major cause of intramammary infections in small ruminants. The *Staphylococcus aureus* is the main zoonotic pathogen, leading to the need to implement control programs in dairy farms. The methods of diagnosis, prevention, and management need standardization and are discussed in this article.

Introduction

In the last decades, more than 95% of the dairy products consumed in the world have been derived from bovine milk, except for the Mediterranean countries, where goat and sheep milk are part of the cultural heritage [1]. Small ruminant dairy products are a vital part of the economies of many countries, especially in the Middle East and the Mediterranean, with well-organized activities in France, Italy, Spain, and Greece, being consumed by more than half the world's population [2,3].

The importance of goat's milk in human nutrition lies not only in the biological value of its nutrients but also in its characteristics of hypoallergenicity, which makes it a differentiated food [4].

Mastitis is a complex disease resulting from the interaction between the agent, animal and the environment, associated with the presence of microorganisms in most cases. It is an important animal health and public health problem, with great economic repercussion in practically every country in the world [5].

Mastitis can be classified as clinical or subclinical. Animals with clinical mastitis may present with edema, increase in temperature, hardening and pain in the mammary gland, in addition to the presence of lumps, pus or other alterations in the physical characteristics of the milk, as well as systemic alterations that may occur loss of the mammary gland and until death of the animal. The diagnosis is performed by the Tamis test, with the visualization of macroscopic milk alterations. In subclinical form, macroscopic alterations do not occur, but alterations in milk composition, not evidencing signs of inflammation in the mammary halves. Subclinical mastitis presents a positive result to California Mastitis Test (CMT) or other indicative tests, confirmed by microbial isolation. It can be detected by direct or indirect counting of somatic cells in milk [6-8].

The most commonly identified etiologic agents are similar to those found in bovine mastitides, such as coagulase-positive staphylococci (CPS), coagulase-negative staphylococci (CNS), *Streptococcus* spp., *Escherichia coli*, *Corynebacterium* spp., *Pseudomonas* sp. and some species of fungi less frequente [9,10].

The caprine and ovine mastitis related to *S. aureus* are the most important for animal and public health, most of them attributed to inadequate hygiene during milking, excessive suckling of the goatling or lamb, contact with contaminated pastures, and pictures of gangrenous mastitis due to the specific toxins responsible for serious lesions in the breast, such as the α-toxin that produces alveolus necrosis [11].

Considering the sources of infection and transmission routes, the mastitis agents were conventionally classified as contagious and environmental. Contagious bacteria are present in the microbiota of the skin of the mammary, mucosal and conjunctival parts of animals, as well as in milking equipment, in cloths used for cleaning and drying the ceilings and in the hands of the milkers. Flies may participate as mechanical vectors of contagious agents, being important in properties with the high infestation, contributing in the transmission of the microorganisms to goats and sheep in the prepartum [12].

Infections by contagious agents occur predominantly during milking, and microorganisms opportunistically invade the mammary gland, causing an immediate inflammatory response. Among the main contagious pathogens are *S. aureus* and *S. agalactiae*, which occur with high prevalence and usually cause subclinical mastitis, of long duration and with the high counting of somatic cells (CSC) [13].

The group of environmental agents is present in organic matter as soil and feces, in the bed of animals, in water and in the air. The infection occurs mainly in the inter-milking period, but may also occur during milking. Fungi, yeasts, algae, and enterobacteria are the main environmental pathogens, especially important in this group E. coli. Despite the lower prevalence of mastitis caused by these agents, they

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generally cause severe, super-acute clinical conditions, which can lead to death [14].

Prevention and control are starting points for the adoption of sanitary measures within the property. These measures applied simultaneously should reduce the rates of subclinical and clinical infections in the herd and are related to the hygienic aspects of the animals, milking, and people involved in milking. Milking hygiene is one of the key points to the success of a mastitis control program [15].

The early detection of animals with mastitis, the treatment of clinical cases, the milking starting from the healthy animals putting to the end those that are in the treatment, the hygiene and disinfection throughout the milking process, with the use of pre and post dipping, proper maintenance of the vacuum and pulse parameters of the milking machines and the drying treatment are the most important measures for the prevention and control of mastitis, besides the disposal of animals with chronic and recurrent mastites [16].

Animal welfare and milk quality are directly associated with health, nutrition and herd management, as well as the training of labor, proper management of facilities and equipment used during milking and transportation to the industry [17].

Etiology

The etiology of mastitis is complex and multivariate (toxic, traumatic, allergic, metabolic or infectious) making it essential to identify the causal agent, both for prevention and control, and for the monitoring of herds [18].

The pathogens identified are *Staphylococcus* spp. (*S. aureus*, *S. caprae*, S. *epidermidis*, *S. saprophyticus*, *S. hyicus*, S. intermedius, S. simulans, S. equorum, S. capitis, *S. lentus*, *S. gallinarum* and *S. xylosus* [4,19,20], *M. capricolum*, *M. putrefaciens* and *S. equi* subspecies ruminatorum [21,22] and less frequently *Mycoplasma agalactiae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Brucella* spp., *Mycobacterium* spp., *Yersinia pseudotuberculosis*, *Coxiella burnetti*, *Mannheimia haemolytica*, *Corynebacterium* spp., *Yersinia pseudotuberculosis* and *Nocardia* spp., As well as fungi and yeasts: *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Cryptococcus albidus*, *Cryptococcus neoformans*, *Rhodotorula glutinis* and *Geotrichum candidum* [10,11,14,18,23], besides the algae [5].

The main pathogens isolated belong to the genus *Staphylococcus* spp. [4,19,24,25,26]. One of the main characteristics of mastitis concerns the diversity of agents with pathogenic potential. Among these, we highlight the CNS, which for other animal species are considered minor pathogens. There are still bacteria of the genus *Streptococcus*, *Corynebacterium*, *Pseudomonas*, *Mannheimia* and some species of fungi, but less frequente [10,27,28]. Some studies with the experimental infection have demonstrated the pathogenic potential of some relevant microorganisms, such as *Corynebacterium pseudotuberculosis*, which is responsible for triggering acute conditions accompanied by changes in the leukogram [29].

S. aureus is the most prevalent agent. In decreasing order of frequency, CNS appear, which are major pathogens in small ruminants, *Streptococcus* spp., *Enterobacteria*, *Arcanobacterium pyogenes* (now *Trueperella pyogenes*), *Corynebacterium* spp., *Pasteurella* spp. and *Pseudomonas* spp. In relation to subclinical mastitis, the main isolated agents are CNS, *S. aureus*, Gram-negative bacilli (GNB), *Streptococcus* spp. and *Corynebacterium* spp. [19,27,28].

Among the prevalent CNS species, *S. caprae* and *S. epidermidis* present a higher frequency of isolation in goats. According to Bergonier et al. [27], studies show a higher occurrence of *S. caprae*, however, *S. epidermidis* is associated, in most cases, with high CSC, and the same fact is not observed for *S. caprae*[18,24,27].

The prevalent CPS species are: *S. aureus*, *S. hyicus*, and *S. intermedius*, and are also associated with outbreaks of food poisoning [30,31].

Staphylococcus aureus is the most pathogenic infectious agent for the mammary gland, both in the form of subclinical and clinical infection, and its importance for public health is evident. The toxins produced cause vascular thrombosis, gangrene and detachment of underlying tissues [10,11,15]. This bacterium produces a wide variety of hemolysins (alpha, beta, and delta), which contribute to bacterial invasion and inhibition of host immune response. Studies have shown that the same host may present different frames in each ceiling. The authors reported a case of *S. aureus* mastitis in a nulliparous goat with two infected ceilings, with the left roof evolving to acute catarrhal mastitis and the right to mastitis with gangrenous detachment [32,33].

Studies to detect enterotoxin A, B and C genes in S. aureus isolates obtained from milk samples from goats and cattle in Brazil showed that the isolates from goat milk with mastitis had high enteropathogenic potential, being superior to those obtained from cattle, in addition, it is suggested that *S. aureus* producers of enterotoxins type C are the main ones involved in the pathogenesis of mastitis [32].

Streptococcus spp. may cause individual mastitis or in the form of an outbreak, with a higher occurrence: *S. dysgalactiae*, *S. uberis*, *S. agalactiae*, and *S. zooepidemicus*. *S. dysgalactiae* and *S. uberis* are the most common causes of mastitis, spreading due to the inadequate hygiene of milkers or milkers. Sporadically, *S. agalactiae* causes mastitis in goats. Infection by this pathogen may result in fibrosis and decrease in milk production, but it is often not associated with systemic signs. The formation of abscesses in ceilings, chronic mastitis and atrophy can be observed in cases of mastitis by *S. zooepidemicus* [11,19,24].

Coliform mastitis appears to be less common in small ruminants than in cattle. The main bacteria causing mastitis by coliforms are *Klebsiella* spp. and *Escherichia coli*. The occurrence is more common in the postpartum period and is associated with severe systemic disease, and may manifest as a persistent or transient infection [34].

Arcanobacterium pyogenes, currently termed *Trueperella pyogenes* [35] can cause infection in non-lactating annals. Development and rupture of abscesses in the mammary gland are typical of this infection. The milk becomes purulent and has a foul odor. Although susceptible to several antibiotics, the cure rate is low due to the formation of abscesses in the breast tissue. Therefore, these animals act as sources of infection in the herd. The affected animals should be discarded to avoid continual contamination of the milking material and/or infection of other animals in the herd [27].

The genus *Pseudomonas* spp. have also been associated with the disease, usually related to poor hygiene of equipment and facilities and/or contaminated water supply. Breast infection may be severe enough to cause systemic symptoms, including septicemia and bloody milk, with *P. aeruginosa* and *P. pseudomallei* being isolated in animals with clinical mastites [36]. In another study, *P. aeruginosa* was associated with the occurrence of purulent mastitis that evolved to gangrene and death [37].

In small ruminants, contagious agalactia is characterized by a pluriethiologic syndrome caused by several mycoplasmas: *Mycoplasma agalactiae*, *M. mycoides* subsp. mycoides and *M. capricolum*. The clinical features are well characterized by a triad of symptoms located in the mammary gland, joints and eyes. There are other species that even without causing this characteristic triad, determine similar clinical alterations, especially mastitis and arthritis: *M. putrefaciens*, and *M. mycoides* subsp. capri [19,20,28,38].

Lentiviruses are also known as infectious agents for small ruminants, however, they are not considered classic mastitis pathogens by the high number of asymptomatic animals. Birgel Junior *et al.* [39] studied the influence of the caprine arthritis encephalitis virus (CAEV) on the physicochemical and cellular characteristics of the milk, being evidenced a significant influence on milk composition, electroconductivity, chloride content and CSC of infected animals.

Currently, the study of mycotic mastitis becomes increasingly important due to the fact that many yeast species, yeast, and filamentous fungi, formerly considered nonpathogenic, have acted as opportunistic agents, causing diseases in animals [40]. Most cases occur in the form of localized outbreaks and/or after antimicrobial treatment [41]. The main genera involved in fungal mastitis in ruminants are *Candida* and *Cryptococcus*, as well as others such as *Geotrichum*, *Pichia* and *Trichosporon*. According to Anderson et al. [11] the pathogens isolated from cases of mastitis in small ruminants are *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Cryptococcus albidus*, *Cryptococcus neoformansanto*, *Rhodotorula glutinis* and *Geotrichum candidum*.

Epidemiology

The triggering of mastitis is influenced by innumerable factors, related to the animal, pathogen, and environment. The factors that influence the susceptibility to mastitis include the natural resistance of the mammary gland, stage of lactation, heredity, an age of the animal, species, infectivity, and pathogenicity of the agente [42].

The sources of infection are with clinical or subclinical mastites and other species of animals that are inserted in the same environment. The pathogens can be eliminated by milk, feces, urine, and oronasal secretions, having as a route of transmission the hands of the milker, milking equipment, vectors, and fomites in a general way. The entry most often is via the galactogenic route and can occur systemically via the hematogenous route. All animals are susceptible, increasing the predisposition mainly according to age and number of lactations [14,24,28,33].

Subclinical mastitis predominates in the herds of small ruminants, presenting prevalence between 5% and 30%. Clinical mastitis is prevalent at levels below 5% and may reach high rates in certain management situations [10].

In goat herds both vertical and horizontal contagion are likely to occur, however, vertical contagion presents very low occurrence. The introduction of mastitis is favored mainly by the factors that intervene in the horizontal transmission of pathogens. This is due to the particularities of the protection of the goat mammary gland, which provides greater resistance to environmental infections due to a higher physiological level of somatic cells in milk, as well as a higher percentage of neutrophil polymorphonuclear cells than the cow [43]. Dohoo and Leslie [44] reported that the goat differs from other domestic ruminants because of its predominantly apocrine type of secretion and that in this species there is a large variation in somatic cell count related to the degree of infection, age, the volume of milk produced and lactation period. The main risk of infection is determined by the microorganisms that colonize the ostium of the ceiling, as well as by the operations that favor the penetration of these through the channel of the ceiling. In this situation, the moment of milking represents a critical point for mastitis control. In addition, one should consider those individual and environmental factors that predispose to the installation of the intramammary infection, in the latter case, closely linked to the operating systems [45].

Among the main determinants of mastitis, it is highlighted that in the lactation period there is a greater susceptibility of the animal to the mastitis of the contagious type, whereas, in the dry period, it is observed a greater frequency of the environmental mastitis. The weaning phase provides a series of changes in the mammary tissue, giving rise to the period of mammary involution [19].

Ameh and Tari [46] studied the predisposing factors for mastitis and found a positive association between mastitis and the presence of injuries in the ceiling, but no association was found between ceiling diameter, the distance between ceiling and soil, and prevalence of mastitis. Moroni *et al.* [47] conducted a study on the risk factors for intramammary infections in dairy goats, and it was observed that mastitis was more frequent among third- and fourth-order birthing females, and as regards the stage of lactation, it was observed higher positivity for animals that had been lactating for several days.

Although the disease manifests itself in different systems of exploration and management, the greater occurrence is in the intensive system, determining a greater predisposition. In the epidemiology of the mastitis two situations are very mentioned: one with an extremely contagious character, which quickly becomes an epidemic in the herd, affecting almost all animals, with a high mortality rate due to septicemia. And another is sporadic, where there is a cyclical tendency of clinical cases that alternate with years of apparent disappearance of the disease. In these cases, the evolution of the disease is very slow and one of the major problems is the decrease of the production and progressive atrophy of the mammary gland, leading the producer to the early disposal of the animals. In relation to the mechanisms of infection, there are three penetration routes that are capable of infecting the animals: digestive, galactophore and respiratory. Other intradermal and subcutaneous routes have also been studied, where mite species that may be vectors of pathogens are suspected [45].

Prevalence indexes of mastitis in the herd help to quantify the health status of the mammary gland of the animals, demonstrating precisely the risk of infection. The estimation of infection risk allows the design of control and surveillance programs, reducing the economic impact caused by the disease [48].

Pathogenesis

In order to establish the infection, the etiologic agent must surpass the terminal portion of the ceiling, since the integrity of the ceiling is the first line of defense [49]. After penetration, the microorganism multiplies in the galactophore channel and reaches the cistern of the ceiling, where it actively multiplies and distributes itself through the mammary parenchyma [15,24,28]. The second line of defense is the immune system that includes leukocytes in the ducts of the ceiling and gland [50,51]. A large contribution of polymorphonuclear leukocytes, particularly neutrophils, is immediately observed as a response of the immune system. The inflammatory process intensifies and the alkalinization of milk secretion is slight due to the extravasation of cellular liquid and ions, such as sodium carbonate and chlorides. They occur due to the great bacterial multiplication: milk acidification, toxin production, tissue damage and the presence of purulent secretion and blood in the milk [15,29,45].

The changes caused by mastitis in the breast tissue are not only reflected in the milk production but also in the physical-chemical characteristics, with alteration of its main components. Mastitis interferes with the composition of milk by the action of leucocyte lipases and, as a result, the fat concentration in the milk coming from ceilings with high CSC tends to decrease [52,53].

At the same time, there are changes in CSC that vary depending on the etiology and virulence of the etiological agent involved in the infectious process [14,24,44]. Somatic cells comprise most of the cells of the mammary gland immune system, in the proportion of 80% in the healthy mammary glands and 99% in the mast cells. These cells are constituents of the natural defense mechanism and include lymphocytes, macrophages, polymorphonuclear leukocytes and some epithelial cells [54,55,56].

Clinical signs and diagnosis

In acute clinical mastitis, fever (40º to 42ºC), edema and firm consistency of the mammary gland, loss of appetite, apathy, dyspnea, enlargement of the retro-mammary lymph nodes and reluctance to move are observed. Claudication is a common clinical sign where small ruminants move their legs so as not to touch the inflamed mammary gland [57]. The milk secretion has an altered staining and may range from the presence of lumps (catarrhal mastitis), pus (apostematous mastitis) to serous or bloody secretion (phlegmonous mastitis) [11,16,28]. However, in subclinical mastitis, the animals do not present clinical signs but alterations in milk composition, showing no signs of inflammation in the ceilings, only positive results to CMT or other indicative tests [57,58].

Goats and sheep present more frequently than other species, the most severe picture of mastitis, gangrenous mastitis. This type of mastitis is caused by *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Clostridium perfringens*. In the initial phase, the animals present a systemic picture with fever followed by hypothermia, inappetence, dehydration, local sensitivity that leads the animal to claudication and reluctance to walk and lie down, congestion of ocular mucosae, tachycardia, hypomotility to ruminal atony, diarrheal feces, weight and even death of the animal. The skin of the mammary gland is erythematous, with increased temperature, edema may be local or even reach the abdominal or xiphoid region, and later become cyanotic [58,59,60] reported that in the present study,

Diagnosis begins with udder inspection by palpation for the detection of abnormalities in the breast tissue, such as the presence of diffuse nodules in the parenchyma, hardened consistency of the gland and local temperature increase, as well as visual inspection of the milk performed by the use of a black background or screened cup for the visualization of macroscopic changes in milk, such as lumps, pus, blood streaks or altered staining [61].

Clinical mastitis can be diagnosed from the clinical signs of the disease; however, the diagnosis of subclinical mastitis can be made using CMT according to Schalm and Noorlander [62], which estimates the number of somatic cells present in the sample. Milk or by counting electronic or microscopic somatic cells. Both evaluate the degree of sanity of the mammary gland since the somatic cells are defense cells of the organism, being constituted mainly by polymorphonuclear leukocytes that migrate from the circulatory chain to the focus of infection in the mammary gland [45].

The CMT is a subjective examination, which must be performed before milking, immediately after the first milk jets are discarded. This is a kit consisting of a racket with four receptacles, where two milliliters of milk is mixed with two milliliters of the reagent that accompanies the kit, which is an anionic detergent, prepared with the bromocresol purple that is a pH indicator, which in the case of alkaline samples, that is, positive, will turn the color of the indicator, showing a violet coloration. The action of the detergent is to lyse the leukocytes releasing the genetic material of the cells, occurring a viscosity proportional to the number of cells present in the milk sample [15,19,24].

According to Schalm and Noorlander [62], the reactions with scoring from a cross, namely: 1+ (formation of lumps), 2+ (discrete gelation) and 3+ (total gelation), similar to catarrh However, Silva et al. (2001) classified the reactions in arithmetic scores as 0: negative (no reaction between reagent and milk), 1: traits (suspected), 2: weakly positive reaction, 3: positive reaction and 4: strongly positive reaction, respectively represented by the average neutrophils per milliliter of milk.

Negative CMT is a good indicator of the non-existence of infections, but positive CMT may not be indicative of infectious processes in the mammary gland. This is due to the presence of epithelial cells in a greater amount when compared to cow's milk, which together with leukocytes, react to CMT leading to a different interpretation of the test used in cattle [63]. An average of 3% of cytoplasmic corpuscles may contain nucleic fragments [43], which together with epithelial cells can apparently raise the concentration of leukocytes present in milk, even in healthy animals. Santos et al. [64] interpreted the reaction of goats' milk to CMT and related the number of somatic cells to the intensity of the reaction. The authors verified that small ruminant without mastitis may show traces or 1+ reactions. On the other hand, 2+ and 3+ reactions may be important indicators of intramammary infection. Smith and Roguinsky [65] classified the milk of the animals analyzed with a score of 1+ as negative for mastitis. It should not be performed in the first two weeks post-partum (colostrum phase) and at the end of lactation (drying period), because in these periods normal physiological changes of the mammary gland occur, leading to a false positive result [34].

Automatic counting of somatic cells can be performed by Coulter Counter® and flow cytometry-based counters (Somacount® or Fossomatic®). The first method is non-specific, based on the electrical impulse count and therefore is influenced by the number of fat globules and cytoplasmic particles, resulting in counts almost twice as large as those of the Fossomatic or Somacount [66]. However, studies differ on the use of Somacount 300®, calibrated for the bovine species, with both positive correlations with direct microscopy [67] and the inverse, with overestimation of CSC [68].

According to Mota [45], CSC forms the basis of the indirect diagnostic techniques of mastitis in all species of dairy ruminants. Polymorphonuclear cells flow into the blood and into the breast by chemotactic effect and in response to inflammatory stimuli. Thus, by means of equipment such as Somacount® and other instruments that perform automated counting of particles by size or staining of the cell nuclei, the intensity of the cellular response, which is related to the type of infection, can be quantified. The normality limit was set at 1.0x10 6 cells/Ml [69]. Studies in Brazil show that CSC variation in milk occurs as a function of a number of factors, including lactation stage, calving order, the season of the year and type of milking [70].

The use of CSC has not yet been well established in the diagnosis of caprine and ovine mastitis. In Brazil, some studies have demonstrated that the maximum, minimum and average values of CSC are very close, being found high values of CSC both in presence and absence of bacterial growth [71].

Silva *et al.* [72] studied the association between CMT and CSC in order to evaluate the health of the mammary gland, and a positive correlation was observed between these two tests. However, high somatic cell counts were observed in samples negative to the microbiological culture, showing that CMT can be used as a screening test in the diagnosis of mastitis in small ruminants, but should always be associated with the microbiological examination.

Direct microscopy, standardized by Prescott and Breed [73], serves as a method of controlling electronic counters, as recommended by the FDA (Food and Drug Administration) when associated with methyl green and pyronin-Y staining, to differentiate nucleated cells from cytoplasmic corpuscles [68].

Direct microscopic counting has been advocated as one of the most reliable methods for determining CSC in goat milk, especially if specific DNA dyes, such as methyl green and Y-pyronin, are used because it differentiates somatic cells from cytoplasmic corpuscles [74]. Dulin et al. [75] analyzed milk from 24 goat females by direct microscopic counting, comparing Wright, hematoxylin-eosin, orange acridine, trypan blue, methyl green and pyronin-Y and Levowitz-Weber dyes; and automatic cell counting (Fossomatic® and Coulter Counter®), finding higher counts when using non-specific DNA methods (Coulter Counter® and Levowitz-Weber dye).

The objective of the microbiological examination is to identify the etiologic agent of mastitis, allowing the antimicrobial sensitivity test to be performed. In order to obtain the milk samples for analysis, both subclinical and clinical cases, it is necessary to proceed with the strict hygiene of the ceilings, by washing with running water, drying with a disposable paper towel, disinfecting the ostium of the ceiling with alcohol 70% or 0.25% iodized alcohol. Scoop the specimen into a sterile flask, after discarding the first jets of milk, to eliminate possible contaminants from the roof channel. The samples should be sent to the laboratory under refrigeration temperature [15,45].

As primary bacteriology, milk samples should be seeded in a medium of 5% sheep blood and MacConkey agar, which allow the isolation of the main pathogens involved in the mastitis and differentiate the enterobacteria from the check of bacterial isolation on MacConkey agar. Concomitant use of Sabouraud-agar dextrose medium added with antibiotics with gentamicin or chlorhexidine allows the isolation of yeasts and fungi, and algae, although the latter are isolated in the blood agar medium with incubation for 48 to 72 hours at 37ºC. The results should be observed every 24 hours for up to 72 hours and evaluated morphologically by the Gram technique [15,19,26].

Sometimes there is a need for special means and conditions for the isolation of pathogens involved in mastitis, such as *Mycobacterium* spp., *Brucella* spp. and *Mycoplasma* spp., anaerobic bacteria, and viruses, however, under the conditions described above, the most frequent pathogens can be isolated. When infectious mastitis is suspected and the microbiological culture proves to be negative, culture media and special isolation conditions should be used. There are also cases where milk cultures may be negative and may be aseptic mastitis, mainly of traumatic or management origin, or intermittent elimination of the agent [15].

The microbiological diagnosis also includes the biochemical characterization of the isolates, allowing the phenotypic and genotypic characterization, as well as the antibiogram for the study of the sensitivity of the agents against antimicrobials, allowing the orientation and indication of the most appropriate treatment. Due to its high cost, the antibiogram should be limited to cases of clinical mastitis, relapsing, suspicion of unusual pathogens or experimental studies [15,70].

The success in the clinical diagnosis of contagious agalactia is due to an adequate anamnesis, considering the process related to the herd. Any decrease in productivity, be it intense or moderate, should be carefully investigated for mycoplasmosis. The types of samples for laboratory diagnosis are blood serum, milk secretion, joint exudate (sample of choice for the detection of carriers) and a swab of joint and ocular secretion [76]. The differential diagnosis is mainly made with caprine arthritis encephalitis. This disease presents a very similar clinical picture, and its differentiation must be carried out in the laboratory by the identification of the agent, characterization of the colonies, biochemical or serological tests [70].

Molecular techniques are important tools in the diagnosis of mastitis, presenting high sensitivity and specificity in the identification of pathogenic microorganisms and toxins in milk. One of the techniques that are being increasingly used for bacterial identification is multiplex-PCR, which uses more than one pair of primers in the same reaction allowing the simultaneous amplification of several DNA sequences. This technique allows the identification of more than one bacterial species in the same reaction, promoting a broader and faster evaluation of the presence of pathogenic bacteria in foods [77].

Besides these, it is also possible to perform other tests such as: analysis of variations in milk composition, which is based on the measurement of ions by electrical conductivity and other parameters such as lactose; analysis of serum proteins in milk that appear to respond to the increase in the vascular permeability of the mammary barrier and can be quantified when they reach the infected mammary gland; and research on specific enzymes indicative of breast tissue lesion such as NAGase, which is an intracellular lysosomal enzyme of secretory epithelial cells that appear in greater amounts in milk when degeneration processes occur as a consequence of inflammation [78,45].

Treatment, prevention, and control

Hygiene during milking is the basis for the success of a program to control mastitis in small ruminants. The hygienic-sanitary management aimed at preventing mastitis involves a number of factors including the choice of antimicrobial, microorganism susceptibility, duration of treatment, dosage employed, and the animal's immune status [11,20,26,70].

According to Domingues and Langoni [16], a broad mastitis control program based on prevention will provide a reduction in losses associated with mastitis, improve milk quality and increase yield. It is mainly based on management and includes the following measures: hygienization of the hands of milkers, facilities, and equipment; to milk first the healthy goats, establishing a line of milking; pre-dipping (wash the ceilings before each milking with disinfectant solution and wipe with disposable paper towel); test the black-bottomed mug or screened daily before each milking to detect acute clinical mastitis, looking for macroscopic changes in milk, such as lumps, pus or blood; post-dipping: after milking, immerse the ceiling in disinfectant solution, iodine-based, chlorhexidine, or citrus seed extract, preferably added glycerin; to perform monthly the CMT test, aiming to monitor cases of subclinical mastitis; all cases of clinical mastitis should be treated as early as possible.

Usually, the intramammary route is used for the treatment. The anti-mastitis agen may be used once or twice daily for 3 to 5 days, depending on the clinical picture presented. The ceilings should be milked thoroughly before applying for the medicine [33,70].

Dry therapy is not a routine practice. However, treatment is recommended after the last milking at the time of drying. This therapy has been shown to be an effective tool in reducing the prevalence of subclinical mastitis in the herd, reducing the occurrence of clinical mastitis in goats and dry sheep, and reducing the incidence of new infections after childbirth, leading to gains in production [79,80].

A limited number of licensed antibiotics are available for use in small ruminants. The indiscriminate use of licensed drugs for cattle in small ruminants is a high risk because safety and efficacy are little known for these species [70]. Some studies carried out in Brazil have shown the sensitivity of different pathogens isolated from cases of mastitis in goats and sheep, mainly for gentamicina [4,24,81].

Antibiotic treatment should be accompanied by a veterinarian, ensuring adequate and hygienic administration [10]. Overuse of antibiotics may increase the risk of resistance to these drugs, a fact that has become a public health problem. Goni et al. [82] have pointed out that the detection of *S. aureus* strains resistant to aminoglycosides should be considered a public health concern gave the similar mechanism for strains isolated in humans. Soares *et al.* [83] demonstrated that clinical isolates of CNS from samples collected from animals and humans have shown high resistance to penicillin and ampicillin.

The use of vaccines is an economic decision for veterinarians and breeders since it reduces costs and has positive effects on milk quality and public health, reducing the need for antibiotics [84]. There are commercially available vaccines against gangrenous clinical mastitis for small ruminants and are indicated in cases of the high prevalence of infection [70]. The effectiveness of mastitis vaccination programs for *S. aureus* has been reported for sheep, but not for goats [85,86]. Coelho *et al.* [87] observed a reduction in the number of mastitis cases in dairy goats from the association between antibiotic therapy and staphylococcal mastitis vaccine.

Vitamin E supplementation is important for the functioning of the immune system, acting on antibody production, cell proliferation, cytokine production, prostaglandin metabolism and neutrophil functions [88]. The mechanism by which this vitamin enhances the defense of the body in infections is attributed to its antioxidant effect that protects the phagocytic cells and tissues surrounding the oxidative attacks of free radicals produced by the neutrophil and macrophages respiratory burst during phagocytosis [89,90]. A study by Paes *et al.* [91] demonstrated that CSC and *S. aureus* numbers are lower in goats supplemented with vitamin E.

Homeopathic or phytotherapeutic therapies are little used in small ruminants in Brazil, being used mostly in the treatment of bovine mastites [92]. Langoni *et al.* [93] evaluated the efficacy of a homeopathic compound in the treatment and prevention of mastitis in goats, mainly by analyzing its effect on CSC and milk production before, during and after its administration, demonstrating that CSC and milk production were not affected by homeopathic compound used.The spontaneous cure is the total disappearance of the disease in the absence of any type of treatment, being closely related to the cellular and humoral response of the immune system, varying the cure rate between 10% and 30% in the herd [34].

For success in the prevention and control of mastitis, it is important to have a program for diagnosis and constant monitoring within the rural property [94].

Public health impact

The main pathogens causing infections and toxins related to the consumption of milk and its derivatives are *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli*, *Mycobacterium bovis* and *Brucella abortus* [45,70,95].

According to Freiras *et al.* [96], *S. aureus* assumes great importance in food microbiology as an agent of food poisoning due to the production of thermostable enterotoxins, serving as a hygienic-sanitary indicator in the food industry. Staphylococcal food poisoning is attributed to the ingestion of toxins produced and released by bacteria during their multiplication in food, posing a risk to public health. The toxin, because it is thermostable, can remain in the food even after heat treatment, favoring the occurrence of intoxication, characterized clinically by nausea, emesis, malaise, general weakness, diarrhea, headache and abdominal pain [97].

Salmonella spp. has been the bacterium most commonly associated with outbreaks of food poisoning in the United States and the United Kingdom [98]. In Brazil, Nero *et al.* [99] detected the presence of *S. enterica* in samples of raw milk. Toxic-food infections caused by salmonella are often associated with the ingestion of meat, poultry, eggs, milk, and derivatives without heat treatment. The genus *Salmonella* spp. has a low prevalence in the case of mastitis in goats and sheep, however, salmonellosis is considered to be the disease most commonly associated with outbreaks of food poisoning. Currently, more than 2.000 serotypes of *Salmonella* spp. Are described, with *Salmonella enterica* serotype Typhimurium being the most prevalent in infections for domestic and human animals. Contamination of milk and its derivatives occurs through cross-contamination in the milking or industrialization of the product. The symptoms in the human species are abdominal pain, diarrhea, fever, chills, headache, malaise and emesis, symptoms that may persist for a week or more, and may be fatal especially in children and the elderly [100].

Escherichia coli presents great importance in public health, mainly by the outbreaks involving serotype O157: H7. It is present in the intestinal microbiota of animals and humans and can cause intestinal infections, urinary infections, septicemia, meningitis, and other infections. The contamination of milk and its derivatives occurs in the same way as in salmonellosis and is not considered an important primary agent of mastites [101].

Tuberculosis in small ruminants is characterized by progressive cachexia, dry, short and repetitive cough, mastitis and infertility, and localized or generalized lymphadenomegaly may occur. It is mainly caused by *Mycobacterium bovis*, although *M. avium* and *M. tuberculosis* have been isolated occasionally. The symptoms in humans are a cough, fever, sputum that in the advanced stage of the disease can present blood, difficulty breathing and progressive weight loss. The lack of reports on tuberculosis caused by *M. bovis* in goats and sheep in our country may be due to diagnostic failures since caseous lymphadenitis (*Corynebacterium pseudotuberculosis*) presents macroscopic lesions similar to those of tuberculosis and is widely distributed in Brazilian farms [102].

Brucellosis is characterized by abortion in the final third of gestation, drop in fertility, stillbirth, and decrease in milk yield [95]. In humans, there is acute or insidious fever, night sweats, fatigue, anorexia, weight loss, headache and arthralgia. The main agent of brucellosis is *Brucella melitensis*, which does not occur in Brazil [103]. However, sporadic infections by *Brucella abortus*, which has the bovine host as the main host, may occur in other species when they accidentally come into contact with the agent of bovine origin [14]. This infection may represent an important source of economic losses for producers because there is no specific treatment and euthanasia of infected animals is recommended [103].

Brucellosis, tuberculosis and food-borne toxic-infections by *S. aureus*, *Salmonella* spp. and *E. coli*, are transmitted to the human species through direct contact with contaminated materials or indirectly through ingestion of contaminated foods, mainly milk and its unpasteurized derivatives [100].

There is a report in Brazil of the occurrence of acute toxoplasmosis in three members of the same family from Belo Horizonte-MG, related to the intake of unboiled or pasteurized goat's milk. Tachyzoites were isolated from the milk of one of these goats by inoculation in mice. The goats were raised loosely in the peridomicile to supply milk. Antibodies to *Toxoplasma gondii* were detected in the fifteen animals examined, of which eight had titers greater than 1024. In five lactating goats, titers ranged from 1024 to 32768. As the tachyzoites are destroyed by gastric juice, it is likely that the parasite has penetrated through the mouth and pharyngeal mucosa [104].

Antimicrobial treatment in dairy herds should alert to the possibility of residues in milk, posing a risk to public health by provoking allergic or toxic reactions in individuals who ingest contaminated milk. Allergic reactions usually manifest as hives, dermatitis, rhinitis and bronchial asthma, and are mainly associated with penicillins, but tetracycline, streptomycin, and sulfonamides may also cause this type of reaction. In pregnant women, there are adverse effects on fetuses such as ototoxicity and altered bone development [105].

Economic impact

In addition, the use of mastitis in the treatment of breastfeeding has led to a reduction in the quality and quantity of milk and its by-products [75,106,107], besides being an important public health problem [24].

Although in small ruminants clinical mastitis is responsible for significant losses, subclinical mastitis presents a higher occurrence, generating high losses in milk production. It is estimated that losses in milk production related to subclinical mastitis can vary from 55 to 132 kg of milk/year and a reduction of 3g of fat/kg of milk per animal [108].

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