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REVIEW ARTICLE

Infectious and Non-infectious Factors Affecting Somatic Cell Count and New Diagnostic Approaches of Intramammary Infections in Dairy Goats: A Review

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Abstract: ***Background*:** Intramammary infections constitute major animal health and economic problem in commercial dairy goat farms, being responsible for decreases in milk yield, alter milk composition, and lower milk quality.

***Objectives*:** This paper reviews the published literature during the last three decades, highlighting the multiplicity of non-infectious and infectious factors that influence somatic cell count (SCC). Besides that, it intends to contribute to understanding the conventional diagnostic methods and their limitations, and supports the implementation of new technologies for efficient mastitis control, including the use of infrared thermography and ultrasonography.

***Methods*:** A search on Medline, ScienceDirect, and University Institutional Repositories databases was performed using "goats, AND mastitis OR intramammary infections OR somatic cells count" for publications from 1990 to present (2020).

***Results*:** A total of 144 publications were selected. The SCC is the most important criteria to evaluate the inflammatory status of the mammary glands in goats, but several non-infectious factors (*e.g.*, phenotypic, reproductive, lactational factors) should be taken into consideration for its interpretation. Bacteria and fungi as well as lentivirus are commonly responsive for intramammary infections. Intermittent secretion or environmental contamination of milk pathogens, costs, and time delay poses challenges using conventional diagnostic methods. Ultrasonographic and thermographic techniques applied to the udder seem to be of diagnostic value in acute and chronic mastitis.

***Conclusion*:** Unlike other ruminants species, non-infectious factors have a major impact on SCC which should be taken in account for mastitis diagnosis, and according to milk pathogens detection. Further research in imagological techniques is needed to accurately contribute to implant new mastitis control strategies.

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1. INTRODUCTION

Intramammary infections (IMI) or mastitis, defined as an inflammation of the mammary gland, is recognized as the major cause of decreases in milk yield and composition changes, causing high economic losses to the dairy industry [1-3]. Mastitis in dairy goats is predominantly subclinical, with a prevalence between 5 to 30%, much higher than the clinical form (less than 5%). Subclinical mastitis causes the most significant impact on farms, characterized by the absence of detectable clinical changes or inflammatory signs [2, 4]. It is in general poorly detected, and most of the infections persist in the mammary gland throughout lactation and even during the drying period, with infected animals serving as reservoirs spreading the infection. Monitoring the udder health plays an important role in diagnosis and in reducing the incidence of mastitis in the flock.

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Somatic Cell Count (SCC) is the commonly used criterion for assessing the existence of IMI in dairy ruminants [5, 6]. The most important reason for SCC increases and variations in milk is the infection status of the mammary gland. However, in dairy goats, non-infectious factors such as breed, parity, stage of lactation or season, may contribute to significant changes in milk SCC [7, 8]. The definitive method of diagnosis of subclinical mastitis in goats is made through the analysis of milk samples by bacteriological culture and microbiological identification [9]. Although this is considered the reference method [2] it requires laboratory support and time for the culture development, besides being associated with elevated costs at a flock level. The measurement of the SCC remains, therefore, and despite the unreliable results, the easiest and common method used for mastitis diagnosis in goats. The implementation of new field diagnostic techniques, easy to perform and without an increase of cost per use for producers, are needed.

Infrared thermography (IRT) and ultrasonography are already being used in dairy cows as potential, accurate and non-invasive methods to detect inflammatory processes. In the early stages of IMI, the inflammatory process is characterized by the dilatation of blood vessels, which increases the blood flow and consequently the local temperature. This temperature rise detected by IRT as infrared radiation may be useful as an early detection method for mastitis [10-12]; and it is earlier than conventional diagnostic techniques [13-15]. Ultrasonography is a complementary means of diagnosis, widely used in different medical areas presenting the advantage of being non-invasive and easily transported. In goats, it has been used mainly at the scientific level, performed to visualize physiological and pathological changes occurring on the mammary gland structures [16, 17]. There are studies related to milking and IMI diagnosis which have been carried out through the measurement of the different gland structures with the use of B-mode ultrasonography [18-20]. Based on the observed results, ultrasonography can be considered as a good auxiliary diagnostic method.

Thus, the present work aims to make a critical review of the main aspects related to the diagnosis of mastitis in a field context.

2. MASTITIS DEFINITION AND CONSEQUENCES TO MILK QUALITY

Mastitis is a general term that refers to the inflammation of the mammary gland in response to physical trauma and mainly due to IMI. This is considered one of the most important diseases in dairy ruminants [1, 3]. It is responsible for important economic losses, associated with reduced milk yield, changes in milk composition and quality, lowering the hygienic value of milk, and impairing cheese profitability. IMI is also associated with reduced weight gain in lambs and meat kids, early culling of animals, besides an increased treatment cost.

Clinically, mastitis can be divided into two categories: clinical and subclinical mastitis. Clinical mastitis is an inflammatory response to infection, causing clearly visible physical changes in udder and milk. Common inflammatory signs are swollen and redness painful udders. The systemic signs of the disease include fever, depression and anorexia. In addition, there is a decrease in milk yield, color, consistency and composition [21]. Depending on the intensity of the symptoms, it is subdivided into hyperacute, acute, subacute and chronic [7].

Subclinical mastitis is characterized by the absence of detectable clinical changes or inflammatory signs. This is the most common, with a prevalence between 5 to 30% in dairy goats, much higher than the clinical form (less than 5%), responsible for the most significant economic impact on farms.

Subclinical mastitis is in general poorly detected, and most of the infections persist in the mammary gland throughout lactation and even during the drying period, with infected animals serving as reservoirs spreading an infection to healthy animals [7].

Although this may not present visible milk abnormalities, it is associated with changes in milk composition, with a significant increase in Somatic Cells (SC) and an abrupt decrease in milk production [2, 4, 22]. The SCC is the commonly used criteria to evaluate the health status of the mammary glands and milk quality in cattle, and therefore, of the presence and degree of IMI. High SCCs normally indicate infection of the mammary gland [23]; however, due to the SCC variations seen in goats, milk samples with high counts may not be equivalent of infection [24]. In goats, it is required that non-infectious factors, physiological and management factors, be considered when SCC is used as an indicator of IMI, as they have a major impact on SCC and help to justify the high variability seen in goats [25].

During intramammary infections, milk from infected animals presents changes in component concentrations that can negatively interfere in the technological processes of milk transformation and cheese manufacture (Table **1**) [26, 27]. It has been determined that there is an increase in total proteins due to a rise in the level of whey protein, IgG and serum albumin concentrations in infected animals [28, 29], with the exception of casein concentrations that seems to suffer no impact with IMI. Lactose concentrations seem to be lower in infected animals, yet, results are conflicting for goats´ milk [29, 30].

Fat is the most important component for cheese manufacture in terms of physical and sensory characteristics, and a decrease in the concentration of this component is also expected during IMI. The growing inflammatory response and the associated reduction on the synthetic and secretory capacity of the mammary gland may justify the low concentrations [31-33].

However, data concerning fat concentration in mastitic goats' milk are conflicting [30, 34-36], probably these are related to the time of sampling during lactation or with the causative agent and its pathogenicity.

3. MILK PATHOGENS

Intramammary infections may have different etiologies. It may be due to physical injury (mechanical or traumatic), have a pathogen origin caused by lentiviruses, fungi [7, 37] , but mainly due to bacterial infections [38].

Table 1. Effect of milk somatic cells count (SCC) on milk yield and composition at 200-days of lactation. Adapted from Crémoux *et al.* (1996) (89).

SCC (10^3 cells mL^{-1})	Results for 200 Days of Lactation			
	400-800	800-1600	1600-3200	>3200
Goats (n)	6070	7841	4291	1054
Milk (kg)	700 (-11%)	660 (-16%)	622 (-21%)	571 (-28%)
Fatty matter (kg)	21.8 (-15%)	20.5 (-20%)	19.1 (-25%)	17.5 (-31%)
Protein matter (kg)	19.6 (-11%)	18.6 (-16%)	17.8 (-19%)	16.6 (-25%)
Fat (g/kg)	31.2	31.1	30.8	30.6
Protein (g/kg)	27.9	28.2	28.6	29.1

Depending on the degree of inflammation produced in the mammary gland, pathogens can be divided into minor or major pathogens [39]. Major pathogens, such as *Staphylococcus aureus*, *M. agalactiae, Streptococcus* spp., *Brucella* spp., *Mannheimia* spp*., Aspergillus fumigatus, Escherichia coli,* or *Pseudomonas aeruginosa,* are capable of inducing exuberant immune responses and symptoms. The infection caused by these agents is often clinical and results in higher SCC in milk. Minor pathogens, such as, *Corynebacterium* spp., or the controversial Coagulase-negative staphylococci (CNS), usually lead to mild forms of the disease with low SCC [5, 40]. CNS is considered as minor pathogens in goats. However, many authors consider it a non-proper classification as their pathogenicity (potential to increase SCC and decrease milk yield) seems to be associated with the virulence of CNS species. Thus, it is proposed by many authors to classify the CNS species individually or consider, as in sheep, the *in vitro* susceptibility of CNS species to novobiocin as classification criterion; as the species that are resistant to novobiocin seem less pathogenic, causing small decreases in SCC and a slight reduction in milk production [38]. On the contrary, the novobiocin-susceptible CNS seems to be responsible for higher SCC, considerable lesions in the mammary tissue, and important economic losses due to the significant milk yield and quality losses [2, 7, 41].

Depending on the risk of infection, microorganisms can be categorized into contagious, environmental or opportunists. The main habitat of the contagious pathogens is the mammary gland or teat epithelium, with infection normally occurring during milking. The infection caused by environmental agents occurs by the contact of animals with contaminated materials. The opportunists have their natural habitat in the animal and human skin and are the main cause of subclinical mastitis in goats [42, 43].

The most frequently isolated pathogens are *Staphylococcus* spp., responsible for both clinical and subclinical cases. Other agents such as *Streptococcus* spp., *Enterobacteriaceae*, *P. aeruginosa*, *Mannheimia haemolytica* and *Corynebacterium* spp., may occur but with a lower prevalence in goats´ mastitis [2, 44].

Staphylococcus species are divided into CNS and Coagulase-Positive Staphylococci (CPS) [45].

The most prevalent isolated bacteria in goats´ subclinical mastitis are CNS (25 to 93%), *S. aureus* (3 to 37%), *Streptococci* (6%), and gram-negative bacteria (3%) [7, 46-48].

CNS may persist in the mammary gland as a subclinical infection for extended periods of time, up to 7 months, having the capacity to cause clinical mastitis. In both clinical and subclinical infections caused by CNS, a significant increase in SCC and severe decrease in milk production has been found [1, 2, 49-51]. Within the CNS species, *S. epidermidis* and *S. caprae* are the most commonly isolated and responsible for persistent subclinical infections during lactation [2, 7, 43, 50, 52].

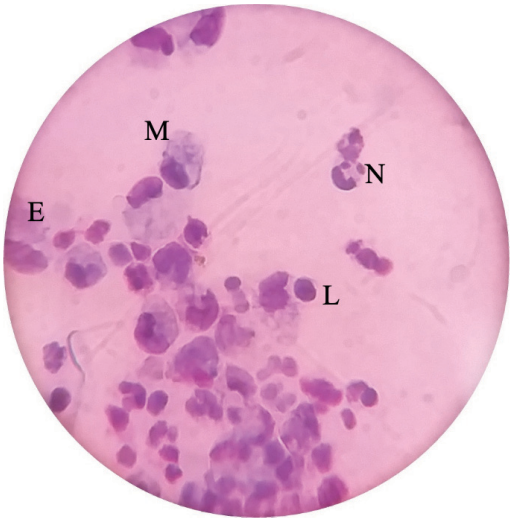
S. aureus is the main aetiological agent present in clinical mastitis cases, followed by CNS, *Streptococcus* spp., Enterobacteriaceae, *Trueperella pyogene*s and *Corynebaterium* spp.

S. aureus is highly contagious and associated with severe symptoms (gangrenous mastitis), beyond also being one of the main responsible for subclinical mastitis. It is highly persistent in the udder due to its ability to produce a protective barrier called “lime” that restricts the efficiency of both the immune response and treatment. *S. aureus* shows dynamic fluctuations and cyclic bacterial shedding in milk that result in SCC fluctuations and lead to false-negative bacteriological results [2, 7, 22, 24, 50, 53, 54]. Another concern about *S. aureus* is the capability of producing thermostable enterotoxins that resist pasteurized milk and can cause intoxication after consumption of contaminated dairy products [2, 52].

After *Staphylococci, Streptococci* is the second most isolated genus in dairy goat farms, with a prevalence of 5 to 10%. In goats, these are usually responsible for the onset of clinical cases, associated with problems of environmental contamination and poor hygiene conditions [2, 47, 55].

Mycoplasma spp. are responsible for clinical contagious agalactia, associated with severe decline on milk production and high SCC, causing incomparable economic losses. The main *Mycoplasma* species involved in the aetiology of mastitis are *M. agalactiae, M. mycoides subsp. capri, M. capricolum subsp. capricolum* and *M. putrefaciens* [46].

P. aeruginosa and *E. coli* are the most frequently isolated Gram-negative bacilli. *Pseudomonas* spp. are considered



M - Macrophages E - Epithelial cells; L – Lymphocytes; N – Neutrophils.

Fig. (1). Polymorphonuclear cells content in goat milk into two plates (x 1,000). The somatic cell counts include macrophages, neutrophils, lymphocytes and epithelial cells. Quick diff staining (photographed by G. Margatho). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

opportunistic agents involved in goats´ mastitis, mostly caused by *P. aeruginosa*. *E. coli* is a gram-negative bacterium, found in the digestive tract and eliminated in the environment, contaminating soil, water, and litters. It has a significantly lower prevalence compared to other bacterial species and is associated with poor hygiene and milking conditions.

M. haemolytica is a Gram-negative bacterium part of the normal nasopharyngeal flora in goats. In immunocompromised animals, due to stress or concomitant infections by other pathogens, it may be isolated in milk, with a lower prevalence.

Gram-positive bacilli are less common in goats. *Corynebacterium* spp. are considered as minor pathogens, normally related to subclinical mastitis in goats and low decreases on SCC. *Bacillus* spp. and *Clostridium perfringens,* although rare in small ruminants*,* have also been isolated from mastitic goats' milk.

In addition to the mentioned bacteria other microorganisms can cause intramammary infections in goats. Caprine Arthritis-Encephalitis Virus (CAEV) is classified as RNA-virus of the *Retroviridae* family and *Lentivirus* genus [56] and has a tropism for the udder. The CAEV infections are characterized by interstitial and clinical indurative mastitis ("hard udder") in dairy goats. Although it does not always result in clinical mastitis, it remains subclinical, and increased SCC has been reported for positive animals [57-59].

Fungal infections, particularly yeast infections, may also be responsible for clinical and mainly subclinical mastitis in goats [60]. The most frequent genera isolated from goats´ milk in cases of mycotic mastitis have been *Candida* spp. (*C. albicans* and *C. tropicalis*), *Cryptococcus* spp. (*C. neoformans*), *Rhodotorula* spp., *Curvularia* spp. and *Aspergillus* spp. (*e.g.*, *A. fumigatus*). Some of the species are seen as major pathogens, and also potentially pathogenic for humans as these appear to survive the treatments during cheese manufacture [61]. They are seen as opportunistic environmental pathogens and are related to poor hygiene conditions and management [62]. SCC in IMI caused by yeasts is normally reported as low elevations, depending on the causative species [51].

4. SOMATIC CELLS

SC is present in milk produced by healthy females and include defense cells, leukocytes (macrophages, polymorphonuclear neutrophils, and lymphocytes) and epithelial cells (Fig. **1**) that result from the desquamation of the epithelium of the alveoli and ducts of the mammary gland [23].

During infection, after being in contact with the pathogens, there is an increased influx of blood leukocytes, mainly neutrophils, recruited through chemotaxis to the site of infection (Fig. **2**), contributing to the significant increase in SCC in milk (Table **2**). Besides being an indicator of IMI, high SCC in milk interfere negatively with cheese quality, sensory, and texture. In cows and sheep, SCC is, in itself, an indicator quite sure of the presence or absence of IMI. However, in goats, SCC is usually high and highly variable [6, 23, 24, 63, 64].

4.1. Somatic Cells Count

The multiplicity of factors that influence SCC and cause great variations and higher counts in goats, makes it difficult to establish a threshold for the presence of IMI in goats [65-67]. The European Union legal limits for cow's milk SCC and for microbiological analysis (total viable mesophiles) are well established [68]. As for goat´s milk, only one criterion is defined, the microbiological plate counts that shall be under 1 500 000 UFC/ml, with no reference to SCC.

The determination of the SCC can be carried out from milk samples taken directly from the tank or samples individually taken from each animal. It can be achieved directly

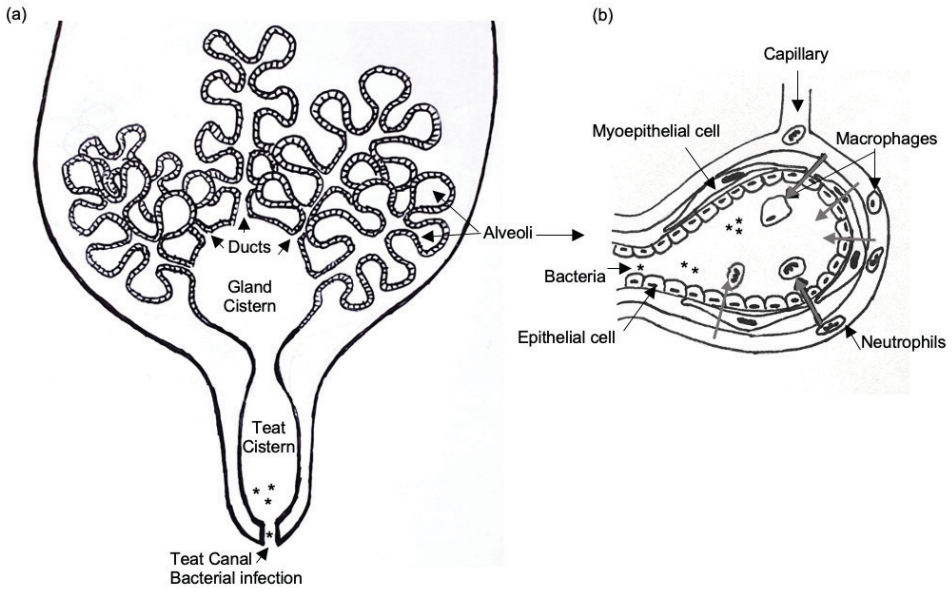


Fig. (2). Schematic representation of mastitis development in an infected udder. **a**) Diagram of a mammary gland illustrating the glandular tissue (alveoli drawn out of scale), emphasizing the entry point for bacteria; **b**) Diagrammatic cross section of an alveolus illustrating intramammary infection and the inflammatory response (original from G. Margatho).

Table 2. Somatic Cell population distribution according to gland health status. Adapted from Jiménez-Granado *et al.* (2014) [23] and Paape *et al.* (2001) [51].

	Healthy Glands	Infected Glands
SCC (103/mL–1)	<750	>1600
CP (103/mL–1)	71 - 306	98 - 231
PMN %	45 - 74	71 - 86%
Macrophages %	15 - 41	8 - 18%
Lymphocytes %	9 - 20%	5 - 11%

SCC - somatic cells count; CP - Cytoplasmic particles; PNM - polymorphonuclear neutrophilic leukocytes.

by microscopic cell counting and automatic counters or indirectly by the widely used California Mastitis Test (CMT).

A direct microscopy, using methylene blue staining is the reference method recommended by the International Dairy Federation [69]; however this method can overestimate the SCC in goat milk because it is not a specific deoxyribonucleic acid (DNA) staining and does not distinguish between leukocytes and cytoplasmic particles. Direct microscopy with a specific DNA staining, the methyl green pyronin [69, 70], that distinguishes between leukocytes and cytoplasmic particles, is the recommended SCC staining in goat's milk. However, this technique is impractical as a routine examination and is only used for experimental studies [32, 51].

Within automatic counters, the Coulter Counter is a non-specific SCC method based on the count of electrical impulses. This method is influenced by the amount of fat globules and cytoplasmic particles present in goats´ milk, resulting in counts almost twice that of the fluoro-optical electronic counters [71, 72]. Fossomatic TM (Foss, Denmark), is an automated DNA specific method based on counting nucleated cells after staining nuclear DNA with a fluorescent dye (*i.e.*, ethidium bromide) [73, 74]. It presents, therefore, comparable results to microscopic counting using DNA-specific stains, as it counts both epithelial cells and leukocytes, and distinguish them from cytoplasmic particles [75, 76]. It provides reliable SCC when calibrated with goat milk in a rapid, repeatable and cheaper procedure than direct microscopy [66-78]. The fossomatic method is usual in most laboratories serving cow milk processors and is normally calibrated using cow milk, which can overestimate SCCs by about 24-34% when used for goats' milk [2, 66, 77, 79].

When it comes to indirect methods, CMT, developed by Schalmand and Noorlander [80], is the most used detection method for mastitis in dairy ruminants, with the advantage of

being animal-side, economical, of easy execution and it gives a very approximate idea of the situation of the herd [71, 81, 82].

CMT estimates the amount of DNA and, indirectly, the concentration of SC in milk. It is based on gel formation and different degrees of viscosity, according to the greater or lesser quantity of SC. The interpretation of results is based on a rating scale ranging of the degree of viscosity, from 0 (negative) to 3+ (positive). The reaction is caused by the presence of a detergent, sodium lauryl sulfate, and bromocresol purple, and the cells present in the milk; the detergent lyses the SC in milk, releasing DNA into the solution and causes gel formation. It has the ability to differentiate the cytoplasmic particles from the goat's milk from the nucleated cells with DNA, and indirectly measures leukocyte concentration in milk [51]. Although not influenced by the apocrine nature of mammary secretion, it is necessary to adapt it to the high cellular basal level found in goats. The CMT score has been shown to have a good correlation with SCC measured with direct methods, when predicting the existence of IMI [44, 71, 82, 83].

Another routine parameter monitored in ruminants, as an indicator of microbiological quality of milk, is the colony count at 30ºC, expressed in the number of colony-forming units per milliliter (CFU/mL). It is an estimation of the milk content in mesophilic aerobic bacteria; however, it does not identify bacteria or possible sources of contamination and does not count all the bacteria present, named psychrophilic ones. For raw milk of small ruminants, the current European regulation [68] sets $1500x10^3$ CFU/mL and $500x10^3$ CFU/mL as the maximum limits for microbiological plate counts, with or without heat treatment, respectively.

4.2. Non-infectious Factors of Somatic Cells Count Variation

In the *apocrine milk secretion* process observed in small ruminants´ mammary gland, portions of cytoplasm from the epithelial cells appear in the milk. Although these Cytoplasmic Particles (CP) do not contain nucleus or DNA, they do contain large quantities of Ribonucleic Acid (RNA) and proteins; besides, these have similar size and morphology to leukocytes and can mistakenly be counted as SC, increasing counts. Compared to sheep's milk, where CP has an average of $15x10^3$ CP / mL, in goat milk the average concentration is $150x10^3$ CP / mL [51]. These higher apocrine component in goats milk secretion is another cause for the high, and variable SCC observed in these animals [51, 64, 84-86].

4.2.1. Stage of Lactation

The most important non-infectious source of SCC variation is the lactation stage. As lactation progresses, SCC increases. This upward trend of SC is negatively correlated with milk production, such that at the end of lactation, the cellular concentration is so high that it is almost impossible to distinguish between infected and non-infected animals through SCC [38, 43, 50].

4.2.2. Number of Lactations

Healthy goats in their first lactation produce less milk [87] and show greater SCC than goats in their third or fourth ones [23, 25, 50, 79, 88]. The influence of the number of lactations on the SCC is highly dependent on the intramammary infections and causative pathogens which the animals have been exposed to during their productive life and damage caused to the mammary gland [89, 90]. In the presence of IMI, older animals show comparable SCC to younger ones [36].

4.2.3. Daily Elevations

Sudden daily elevations ranging from 200 x 10^3 to 2000 x10^3 SC/ mL have been reported, from one day to another, several times during lactation [79]. These increases in SCC can be worrisome since the threshold for an infected animal is under 2000 x10^3 SC/ mL for many authors [25, 89, 91]. Variations throughout the day can also be seen, generally higher in the afternoon and lower in the morning, although it may be associated with a dilution effect, as the milk obtained in the morning milking is higher than in the afternoon [25, 89].

4.2.4. Breed

There are differences in SCC thresholds for infected and non-infected glands (Table **3**) between the different goat breeds around the world, which suggests genetic implications, different management characteristics, or different levels of production. In this sense, some authors attribute lower counts to purebred herds, as their dairy farms tend to be more organized and have more technology [41, 79].

4.2.5. Multiple Births

Milk from animals with multiple births show higher SCC than animals with single birth [23], although animals with multiple births (twins and triplets) produce more milk and present longer lactations compared to animals with single births [33, 92]. Considering that some authors found no influence of prolificacy on SCC, the time of sampling/stage of lactation may be relevant to understand these different results [93].

4.2.6. Milk Contents

The relationship between SCC and milk contents is ambiguous in dairy goats as it could also be associated with a dilution or concentration effect, dependent on the month of lactation, similarly to SCC [28, 33]. For lactose concentration, a negative correlation with SCC has been shown. Studies conducted on the relation between fat content and SCC, described both positive [94, 95], negative [96], or even no relationship [33, 97-99]. Likewise, protein content in milk shows an unclear relationship with SCC. Some authors describe decreased protein associated with higher levels of SCC [85, 100], while others describe increased protein with higher levels of SCC [28, 35, 98] or no relation between both [33, 101].

4.2.7. Udder Morphology

In what concerns udder morphology, some traits, such as udder volume or/and distance between teats are positively correlated with milk yield [102]. Although most studies

Table 3. Somatic cell count (SCC) values of healthy mammary glands in different goat breeds.

Breed	Somatic cell counts (10^3 cells mL^{-1})	References
Alpine	591-887	Calderini *et al.* (1996) [111]
Saanen	585 - 622	
Hungarian white	693-1120	Csanadi *et al.* (2015) [142]
Murciano-Granadina	603-1307	Sánchez *et al.* (2005) [143]
Serrana	750-1200	Mendonça *et al.* (2004) [91]
Damascus	599 - 727	Yakan *et al.* (2019) [144]
Nubian	500 - 600	Paape *et al.* (2007) [6]
Lamancha	450 - 550	
Oberhasli	400 - 500	
Toggenburg	650 - 700	

relate morphological parameters to milk production ones, few authors suggest a possible decrease in SCC through animal udder traits selection [103]. Montaldo and Martínez_lozano reported lower CMT values for globular udders and non-balloon teats, most likely correlated with the greater milking rate of globular udders [104]. On the other hand, it is known that certain mammary gland conformations have a greater propensity for post milking wounds which serves as a gateway to pathogenic microorganisms and intramammary infection, also increasing SCC in milk [50, 105].

4.2.8. Seasonality

Seasonal SCC variation is due to the binomial photoperiod-temperature influence on milk yield and indirectly on SCC [8]. Factors such as grazing fodder offer, temperature, and kidding season have an influence on milk yield and composition. In the spring season, with extended day photoperiod, mild temperatures, and increased food offer, the animals tend to have a longer lactation period, with increased milk production and therefore reduced SCC. On the opposite, during autumn and decreased photoperiod, SCC tend to be higher. In summer, with the highest temperatures and decreased milk production, higher SCC is expected. Moreover, breeding season plays an important influence on the milk yield curve and, therefore, in the variation on SCC, especially with animals under grazing extensive systems [8, 23].

4.2.9. Milking Technique

The studies conducted on the influence of manual or machine milking on SCC are controversial, probably due to animal factors, age, lactation stage, the prevalence of subclinical mastitis, *etc.* [23]. However, recent studies did not find differences between methods, although they found a greater risk of IMI with mechanical milking [23, 38, 41, 106].

4.2.10. Unbalanced Nutrition

Metabolic disorders caused by unbalanced nutrition (acidosis, alkalosis, *etc.*) interfere negatively with milk yield, also increasing SCC [57, 107, 108]. Studies conducted to understand the effect of feed in goats revealed that balanced diets show a significantly lower SCC, as well as diets based only on grazing [107, 109].

4.2.11. Stress Factors

Spontaneous elevations of SCC in goats were also associated with several stress factors. Male introduction during the mating phase [110-112], clinical procedures such as blood sampling, vaccination or intradermo tuberculization [43, 57], and parasitic infestations of scabies [113] have been described as responsible for significant temporary increases in SCC.

5. BACTERIOLOGY

Considering the limitations of SCC methods in goats, the definitive diagnosis of IMI is made through bacterial culture and microbiological identification on milk samples [9], both monitored as indices of milk hygienic quality. The microbiological quality is assessed through raw milk samples collected under aseptic conditions, eliminating the first jets of milk. These samples are cultured and incubated in agar counting plates for 24-72 hours, allowing the count of observable colonies expressed in UFC / ml [2, 9].

Bacteriology is seen as the most reliable method to identify IMI, however, it cannot be seen as the perfect golden standard. Beyond the laboratory support requirement and the costs associated with flock scale, subclinical mastitis may not be accompanied by pathogen isolation. Some bacteria are known to be shed intermittently or shed in too low amounts that may not be present in a milk sample [114] or even present in the milk sample but not detectable using conventional culture methods [49, 115, 116]. On the contrary, it may give false positives when bacteria present in the teat canal or teat skin or present as contaminants are isolated, without signs of inflammation or high SCC [117].

Even being practical, unexpansive and easy to perform as routine in farm conditions, SCC has proved to have some

limitations in goats´ mastitis diagnosis. Although correlated to IMI, several non-infectious factors contribute to variations on SC, requiring that the physiological factors be considered for its interpretation at an individual level [6, 25].

Therefore, the major parameters commonly used to monitor udder health, microbiological count plate, pathogen isolation, and SCC, might fail as indicators of the existence IMI in goats when not used in an integrated manner.

Other field diagnostic methods are needed to obtain unbiased estimates of SCC, and to establish a diagnosis. Also, understand the relationship between the non-infectious parameters that most affect SCC, and integrate them, is crucial to ensure a correct evaluation of routine tests. Developing and implementing new techniques and technologies for mastitis would improve the economic results of dairy goat farms.

6. CAN IMAGING TECHNIQUES BE SUITABLE FOR THE EVALUATION OF MAMMARY GLAND INFLAMMATION IN FIELD CONDITIONS?

6.1. Ultrasonography

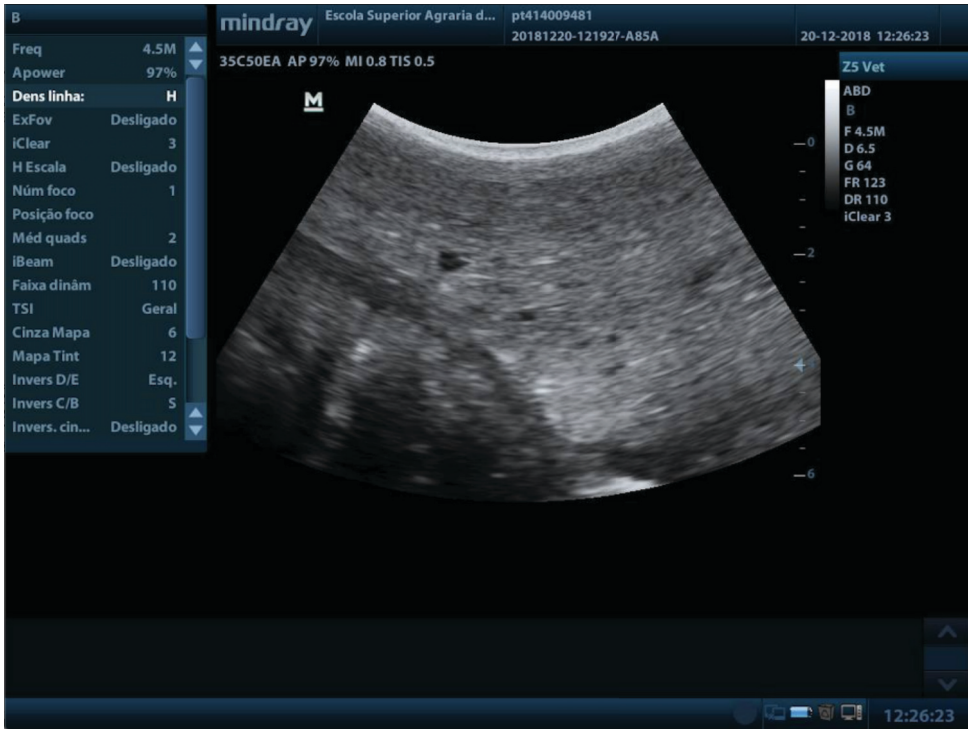
Brightness modulation mode (B-mode) ultrasonography is a real-time diagnostic method. It is widely used in veterinary medicine, as it is a fast, non-invasive, non-ionizing and painless technique [118]. The two-dimensional grayscale image obtained is the resulting reflection of the ultrasound waves emitted from the transducer, after their penetration into the body and at varying degrees depending on the acoustic impedance of the analysed tissues (echogenicity) [119, 120]. The echographic image of the udder in cases of mastitis is affected by the degree of structural changes in the tissue (severity of inflammation and fibrosing) during the course of the disease, and the type of the etiological agent involved echogenicity and echographic homogeneity increase. When structural changes in the parenchyma occur, such as loss of viable tissue and fibrosis, due to an exuberant inflammatory process and increased connective tissue growth.

Through a quantitative assessment, it could be possible to assess a degree of inflammation by assigning a numerical value to echogenicity [17]. The interest in ultrasonography for IMI diagnosis in small ruminants has been growing, as it can be indicated for the diagnose of pathological changes, such as inflammations, obstructions and stenosis, haematomas, abscesses, fibrosis, neoplasia, penetrating foreign bodies, *etc.* [121]. According to several researchers, a healthy goat mammary parenchyma is imaged (B-mode ultrasonographic) as a homogeneous, hyperechoic structure, with anechoic zones, corresponding to parts of blood vessels or lactiferous ducts; the teat canal is seen as a dense white hyperechoic line, surrounded on both sides by parallel dense hypoechoic zones; and the sinus lactiferous is imaged as an anechoic to the hypoechoic structure at the lower part of the gland [122-124]. In the presence of clinical IMI, previous studies revealed qualitative changes in parenchymal homogeneity and echogenicity, with low mean pixel values for healthy glands and higher echogenicity for infected ones [16, 121, 125-127]. In chronic indurative mastitis, a loss of the parenchyma's normal granular pattern and a lack of the anechoic milk canals can be observed, with the presence of hyperechoic structures, representing the increase in connective tissue [119, 128]. A study conducted in goats, performing udder ultrasonography in animals with subclinical mastitis, revealed that this technique was not capable of detecting structural changes in the parenchyma echogenicity [129], which is also confirmed by the authors of this review (Margatho 2020 unpublished data; Fig. **3**).

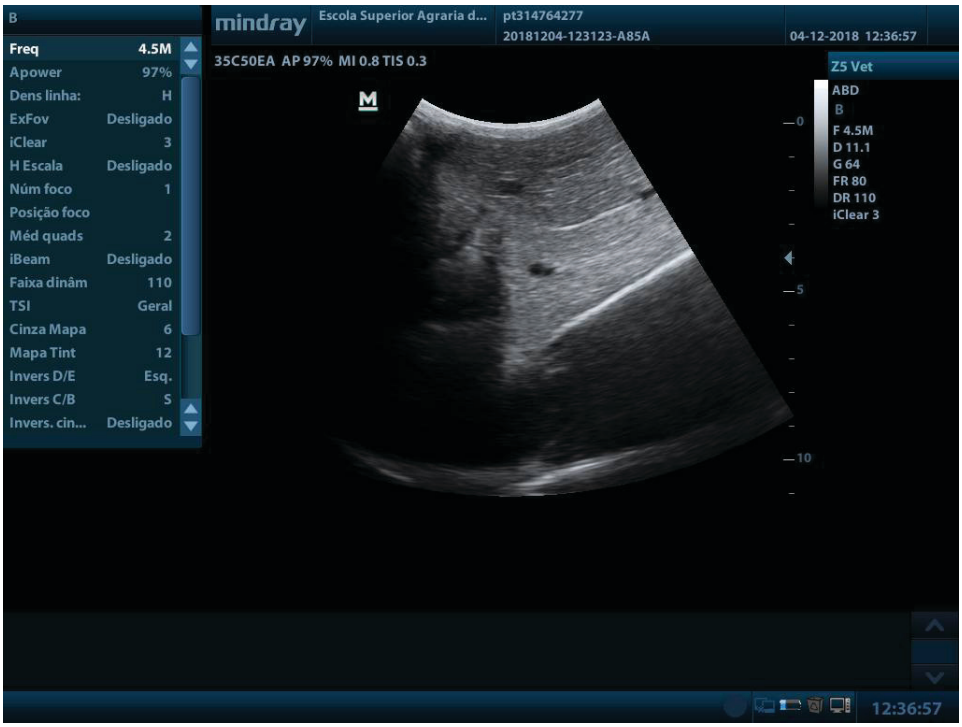
6.2. Infrared Thermography

IRT is a technique that consists of measuring body surface temperature, above the absolute zero, emitted as electromagnetic radiation (according to Boltzmann laws) [130]. IRT absorbs the emitted infrared radiation and generates pictorial images, where the intensity or the color of each pixel is proportional to the corresponding temperature. The formed thermogram provides information about temperature through visible colors to human eyes. The warmest region appears as white or red in a thermogram, whereas the coolest region appears as blue or black. IRT can detect minimal body surface temperature variations in a simple, effective, and non-invasive way. The surface temperature variations reflect the underlying circulation and tissue metabolism, enabling the identification of inflammatory processes that are accompanied by changes in blood flow (local vasodilatation dissipated as infrared energy) and consequently local or systemic infections [131]. During the acute phase of the intramammary infection, one of the inflammatory responses observed is the increase in udder surface temperature; which may be detected by IRT as infrared radiation when other clinical signs are absent and when the conventional diagnostic methods cannot detect any alteration [10-12, 132]. Many studies in dairy cows have focused on the use of IRT for assessing udder health as a potential indirect and non-invasive tool for identifying inflammatory processes accompanied by changes in blood flow [13, 133-136]. With the progression of the process, inflammatory signs tend to disappear, blood flow to the organ returns to basal, the edema decreases, and consequently surface temperature decreases to normal temperature [137, 138]. The chronicity of the process can lead to function loss, with decreased tissue metabolism and reduced blood flow to the infection site, leading to temperatures even above the normal body temperature [136, 139].

IRT potential as an early detection method for mastitis can be of great diagnostic value, compared to the conventional methods [13-15]. In ewes, lower temperatures are reported for clinical and subclinical infected glands [139]. These researchers also detected temperature variation due to changes in the consistency of gland tissue with clinical mastitis, indicating different stages of the inflammatory process. Halves with decreased consistency and small nodules presented the lowest values. On the contrary, Martins *et al.* [140] found higher surface temperatures for the subclinical mastitis group and that temperatures clearly differentiated the subclinical mastitis groups from the others, believing that IRT should be used as a diagnostic technique for subclinical IMI [140]. However, in goats, few studies have been undertaken to approach udder health, and most being related to milking machines [141]. In a study conducted on goats in our laboratory (Margatho unpublished data; Fig. **4**) using IRT as a mastitis diagnostic tool, it was found that the different pathogens cause different inflammatory reactions and cause

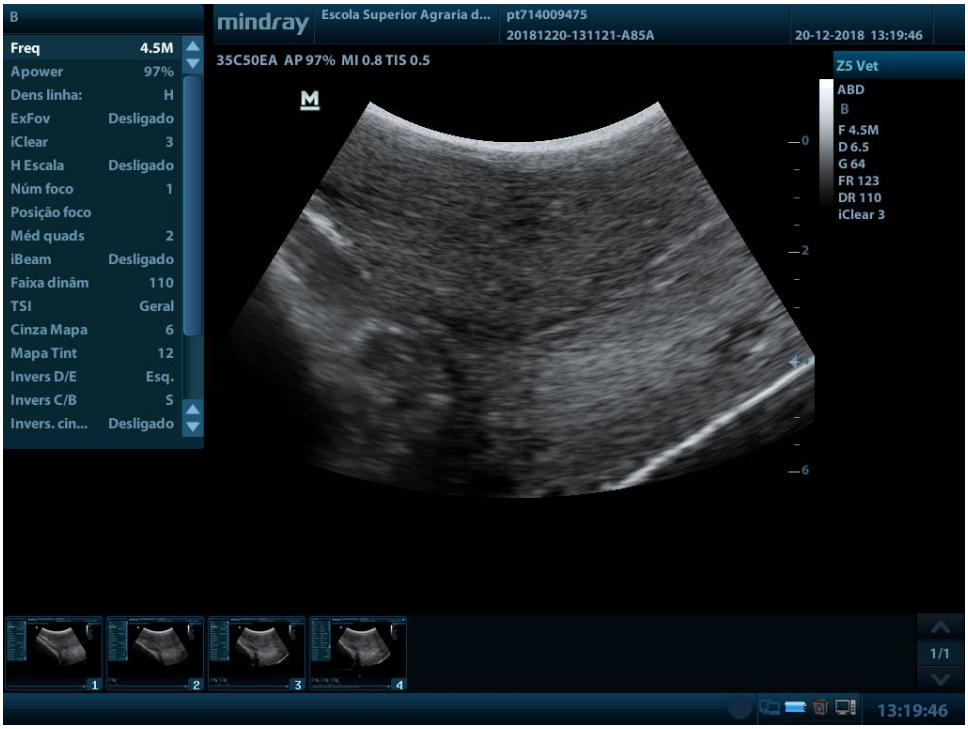


(**a**)

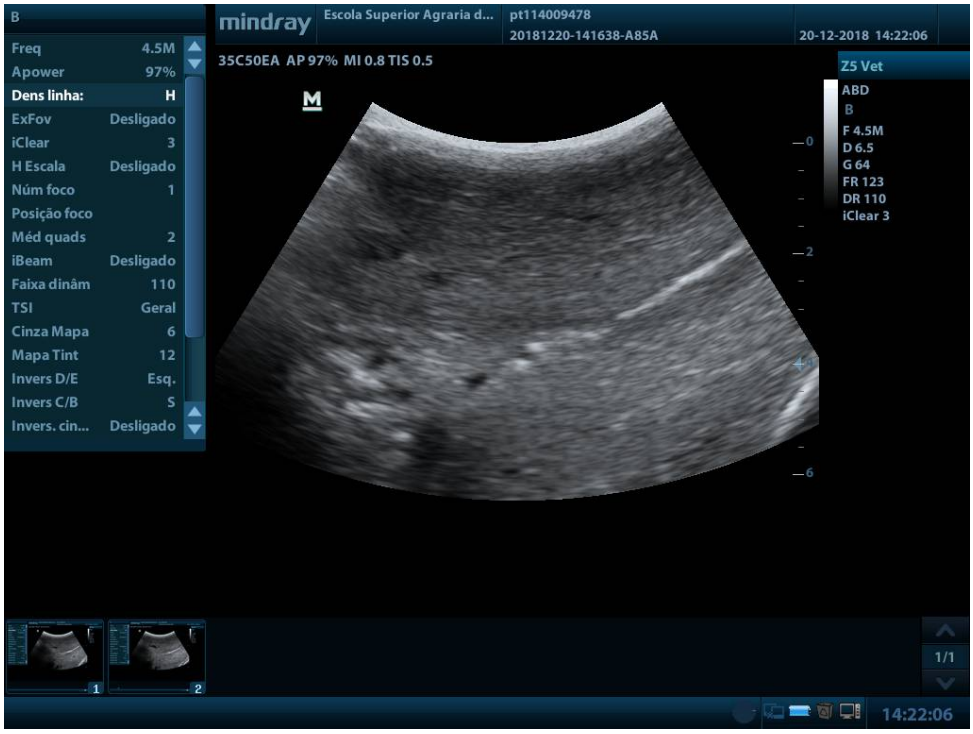


(**b**)

Fig. (3). contd…

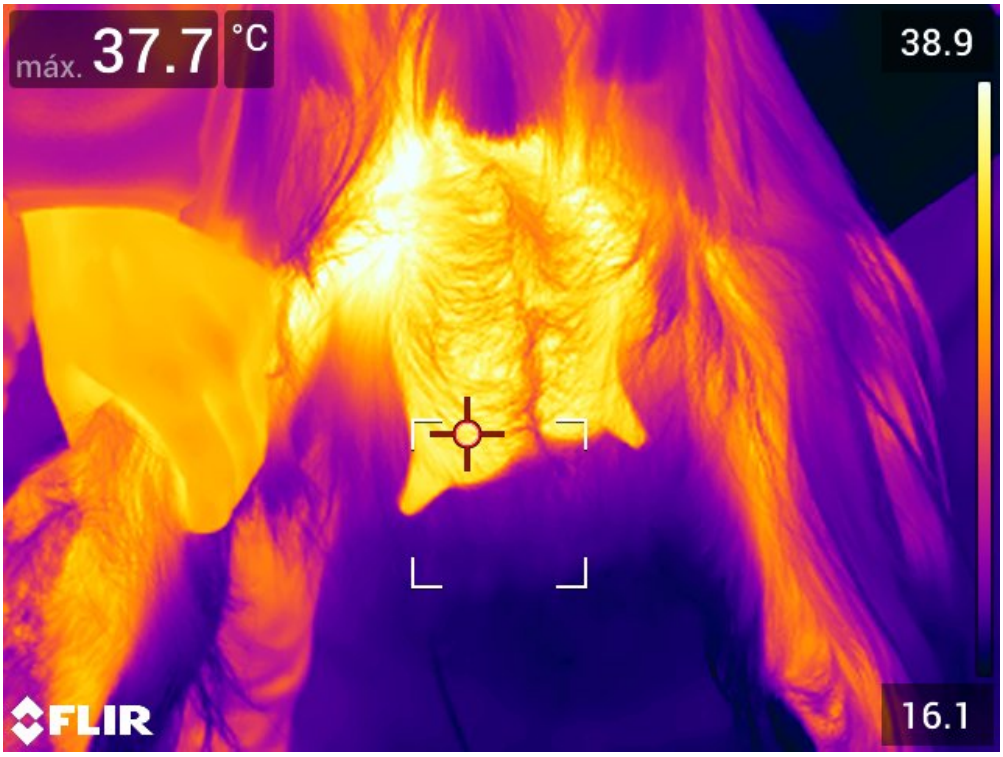


(**c**)

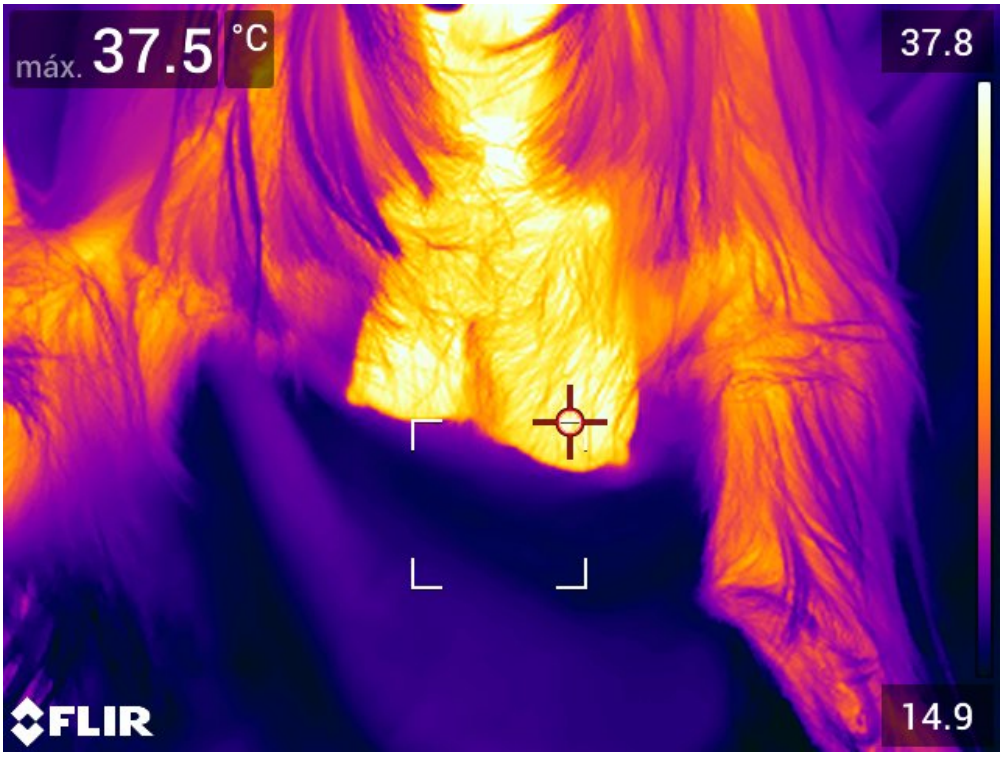


(**d**)

Fig. (3). Ultrasonographic images of udder parenchyma with intramammary infection with the same pathogen on both halves. Gray-scale levels, expressing mean and pixel heterogeneity numerical pixel values (NPV) of each region of interest. (**a**) Negative: 70.5 mean NPV and 19.3 NPV heterogeneity (right); 106.1 mean NPV and 27.8 NPV heterogeneity (left). (**b**) *S. aureus*: 69.8 mean NPV and 19.1 NPV heterogeneity (right); 104.4 mean NPV and 20.9 NPV heterogeneity (left). (**c**) CNS: 66.6 mean NPV and 18.4 NPV heterogeneity (right); 55.2 mean NPV and 16.0 NPV heterogeneity (left). (**d**) *Streptococcus* spp.: 92.1 mean NPV and 25.4 NPV heterogeneity (right); 74.2 mean NPV and 26.8 NPV heterogeneity (left). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

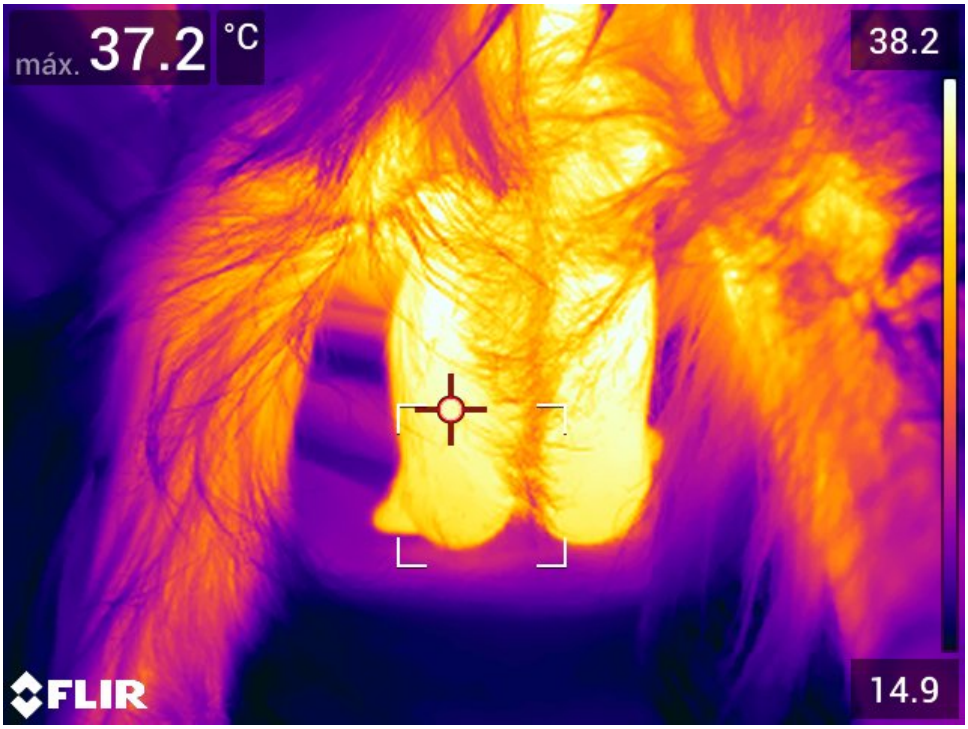


(**a**)

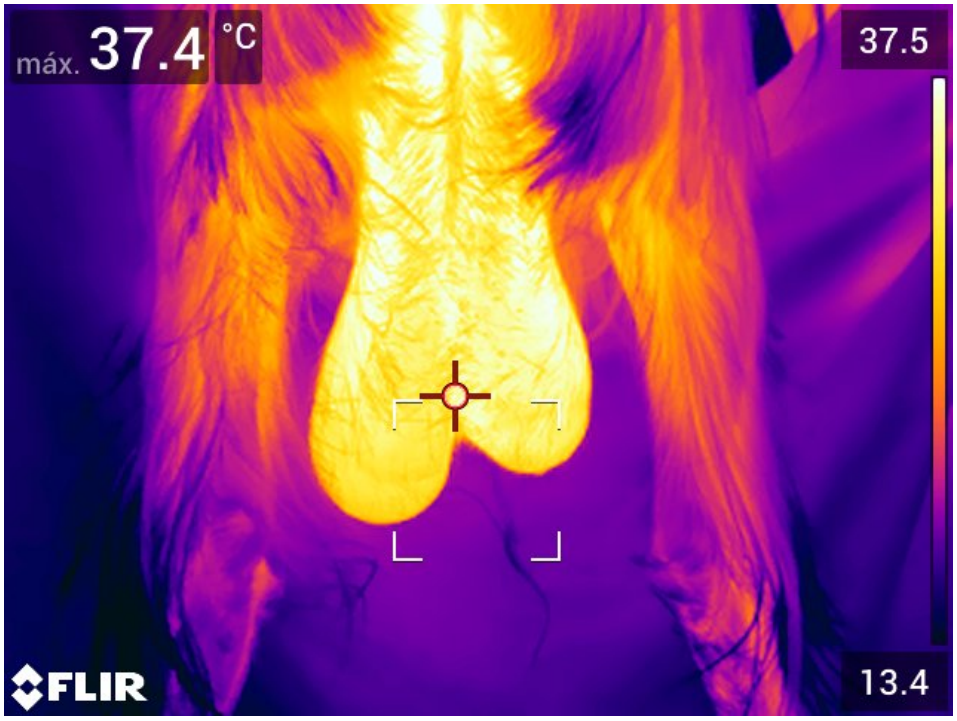


(**b**)

Fig. (4). Contd…



(**c**)



(**d**)

Fig. (4). Infrared thermography (IRT) imagens from udders infected by the same pathogen on both halves. (**a**) Negative. IRT mean: 34,2ºC (right), 34,7ºC (left); IRT teat: 33,9ºC(right), 33,9ºC (left); Somatic cells count (SCC): 1172 x 10^3 and 2995 x 103 SC/ml in right and left halves, respectively. (**b**) *S. aureus*. IRT mean: (35ºC (right), 33,8ºC (left); IRT teat: 31,4 ºC (right), 32,8ºC (left); SCC: 10,887 x 10^3 and 6941 x 10^3 SC/ml in right and left halves, respectively. (**c**) Coagulase negative staphylococci. IRT mean: 36.2ºC (right), 36.5ºC (left); IRT teat: 33.6ºC (right), 34.1ºC (left); SCC: 31,330 x 10^3 and 34,122 x 10^3 SC/ml in right and left halves, respectively. (**d**) *Streptococcus* spp.: IRT mean: 36.3ºC (right), 35.7ºC (left); IRT teat: 34.2ºC (right), 33.6ºC (left); SCC: 18,361 x 10^3 and 6848 x 10^3 SC/ml right and left halves, respectively. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

different acute or chronic mammary damages. It was also observed that in chronic infections, with the decrease in local inflammation, and possible loss of viable glandular tissue, there is a decrease in vascularization, lowering the underlying temperature, even below normal body temperature values, making it difficult to differentiate between healthy and infected glands.

CONCLUSION

In goats, it is required that physiological non-infectious factors, dependent on the animals or not, be considered in SCC interpretation as an indicator of IMI. These non-infectious factors have a major impact on counts and justify the high variability seen in this species [142-144]. On the other hand, the different inflammatory reactions, depending on the microorganism involved in the IMI, make subclinical mastitis diagnosis difficult if only SCC is used. Bacteriology, the gold standard for mastitis diagnosis, has limitations because of the requirement for laboratory support, time for culture to occur, besides elevated costs at the herd level. Hence, the implementation of new diagnostic techniques to access udder health status is needed.

Ultrasonography may serve as a means of excluding other pathologies and for differentiation of structures identified in the parenchyma (abscesses, calcifications, tumors, *etc.*), being a valuable technique when used as a therapeutic aid along with conventional mastitis methods of diagnosis.

Besides, thermography has a great diagnostic potential in the early stages of IMI. Recent infections produce an exuberant inflammatory process that can be observed through an increase up to 2ºC in udder surface temperature. Therefore, the development of a thermographic automated monitoring system, scanning udders and teats daily in milking parlours, alerting to potential increases of temperature, may provide useful information related to recent infections and be efficiently used by the producer or veterinarian.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

CONTRIBUTION OF THE AUTHORS

All authors contributed equally to this manuscript.

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