ORIGINAL ARTICLE

https://doi.org/10.21708/avb.2023.18.3.12014

Assessment of intramammary infections and antibiotic sensitivity in goats from Queimadas, Paraíba, Brazil

Estudo das infecções intramamárias em cabras do município de Queimadas, Paraíba, Brasil

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ABSTRACT: The state of Paraíba is a benchmark in Brazil for goat's milk production. Mastitis is a common disease in dairy herds, often caused by bacteria, and its identification is crucial for promoting efficient treatment. This study aimed to investigate the milking behaviour of goats in the city of Queimadas (Paraíba, Brazil), identify the bacterial species present in the mammary glands of these goats using the MALDI-TOF MS technique, and carry out sensitivity tests on the isolated bacteria. Milk samples were taken from 57 animals across 8 herds, totalling 113 udder halves. After identification, the bacterial colonies were subjected to sensitivity tests. All the farms visited employed hand milking, with all the milk producers washing their hands before starting. Only one milk producer performed the strip cup test and pre-dipping, while four producers performed post-dipping. Bacterial growth was detected in 15 of the 113 udder halves collected, identifying species such as *Staphylococcus caprae*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Micrococcus luteus*. The bacterial isolates were largely susceptible to the antibiotics tested; however, one strain was found to be multi-resistant. The dairy goat herds in the city of Queimadas are reared using systems of incipient technology. Subclinical mastitis occurs in these herds, with the bacteria found being sensitive to various antimicrobials, although resistant strains were also identified.

KEYWORDS: antibiogram; Paraíba Cariri region; lactoculture; goat mastitis.

RESUMO: O Estado da Paraíba é referência no Brasil quanto à produção de leite de cabra. A mastite é uma enfermidade corriqueira em rebanhos leiteiros, muitas vezes causada por bactérias, cuja identificação é importante para promover um tratamento eficiente. O presente estudo objetivou investigar o manejo de ordenha de cabras da cidade de Queimadas-PB, identificar as espécies de bactérias existentes nas glândulas mamárias de cabras pertencentes aos rebanhos utilizando a técnica MALDI-TOF MS, bem como realizar testes de sensibilidade das bactérias encontradas. Colheram-se amostras de leite de 57 animais de 8 rebanhos, totalizando 113 metades mamárias. Após a identificação, as colônias bacterianas foram submetidas a testes de sensibilidade. Em todas as propriedades visitadas verificou-se realização de ordenha manual. Todos os produtores participantes lavam as mãos antes de iniciar a ordenha. Apenas um produtor participante faz o teste da caneca de fundo escuro e pré *dipping*, já o pós *dipping* foi registrado por 4 produtores. Houve crescimento bacteriano em 15 amostras das 113 metades mamárias coletadas, sendo identificadas as espécies *Staphylococcus caprae*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* e *Micrococcus luteus*. Os isolados bacterianos foram amplamente susceptíveis aos antibióticos testados, entretanto, constatou-se uma estirpe multirresistente. Os rebanhos de caprinos leiteiros do município de Queimadas-PB são criados em sistemas de incipiente tecnificação. Nesses rebanhos, há ocorrência de mastite subclínica. As bactérias encontradas revelam-se sensíveis a diversos antimicrobianos, mas foram constatadas estirpes resistentes.

PALAVRAS-CHAVE: antibiograma; Cariri Paraibano; lactocultura; mastite caprina.

INTRODUCTION

The state of Paraíba is renowned for its goat's milk production in Brazil, being recognised as the largest national producer (Delgado Júnior; Siqueira; Stock, 2020). According to the IBGE (2017), the semi-arid region of Paraíba accounts for most of the state's production, approximately 5,481,000 liters per year, with the Cariri region being the largest producer. Here, goat farming plays a significant socio-economic role.

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Received: 01/26/2024. Accepted: 04/29/2024

Mastitis is one of the most prevalent and detrimental diseases affecting dairy goat herds (Lima *et al.*, 2018). This disease involves inflammation of the mammary gland, typically caused by microorganisms, adversely affecting animal welfare, milk production and quality, treatment costs, and posing health risks to both producers and consumers (Peixoto *et al.*, 2016; Hoekstra *et al.*, 2019).

Mastitis is a complex disease with various causes and origins, but it is predominantly due to the contamination of the mammary gland by bacteria such as *Streptococcus agalactiae*, *Escherichia coli*, *Staphylococcus aureus* (Langoni *et al.*, 2017), and various other species of the *Staphylococcus* genus, collectively referred to as non-aureus staphylococci (NAS). Unlike in cattle, NAS hold significant epidemiological importance among goats and sheep, being the primary cause of clinical and subclinical mastitis (Koop *et al.*, 2012; Gosselin *et al.*, 2018).

NAS exhibit great biological variety and physiological diversity, leading to specific infection characteristics in the mammary gland, many of which remain unclear. However, phenotypic tests for species-level identification may lack precision. The Matrix-Assisted Laser Desorption/Ionisation Time of Flight (MALDI-TOF MS) identification technique enables accurate identification of these species and others that may colonise the mammary glands (Gosselin *et al.*, 2018).

Due to its epidemiological and economic significance in dairy farming, clinical mastitis often leads producers to use antibiotics indiscriminately, potentially resulting in the development of increasingly multi-resistant bacterial strains (Lucas *et al.*, 2021). Therefore, precise identification of the bacterial species in the mammary glands, along with bacterial sensitivity tests to the antibiotics used by producers, provides crucial information for effective treatment and proper animal management, promoting human and animal health (Aragão *et al.*, 2021).

This study aimed to investigate the milking practices of goat farmers in Queimadas (Paraíba, Brazil), identify the bacterial species found in the mammary glands using the MALDI-TOF MS technique, and perform sensitivity tests on the identified bacteria

MATERIAL AND METHODS

This research was approved by the Ethics Committee for the Use of Animals (CEUA/UFPB) under number 6968030221.

The study involved 8 producers from the Association of Goat and Sheep Farmers in Queimadas (Paraíba, Brazil). Milk samples were collected from 57 animals across 8 herds, totalling 113 udder halves. During the collections, producers were interviewed about their animal management practices, the occurrence and treatment of mastitis, and their use of antibiotics in goat milk production.

The sample collection process began by extracting the first three milk jets from each udder half for the strip cup test, to check for clinical mastitis signs, such as lumps or abnormalities in the milk. Subsequently, the teat tip was aseptically wiped with a cotton pad soaked in 70% alcohol, followed by the collection of approximately 5 ml of milk from each udder half into sterile tubes. The samples were then stored in cooler boxes with ice and transported to the laboratory, where they were frozen for later lactoculture.

From each sample, 10 µL of milk was collected with a platinum loop calibrated according to Oliver *et al.* (2004) and inoculated onto Petri dishes with agar containing 5% defibrinated sheep's blood. These were incubated in a SOLAB SL101 bacteriological incubator at 37°C for 24 hours. Positive samples were identified by their morphological characteristics, and the bacterial isolates were further cultured on Petri dishes with brain-heart infusion (BHI) agar for identification by mass spectrometry, Matrix-Assisted Laser Desorption/Ionisation - Time of Flight (MALDI-TOF MS), as described by Braga *et al.* (2018).

For the sensitivity tests, isolates were plated onto Petri dishes with BHI agar and incubated at 37°C for 24 hours to re-establish the microorganisms. Bacterial colonies were then sampled using a sterile swab and diluted in 0.85% saline solution. To standardise bacterial multiplication in the inoculum, the McFarland scale was used, following the turbidity standard of the 0.5 tube, which corresponds to an estimated $1.5x10^8$ bacteria. After preparing the bacterial suspension, it was spread onto Petri dishes with Muller Hinton agar. Antibiotic-impregnated discs, specified in Table 1, were placed on the plates, which were then incubated at 37 °C for 24 hours to measure the inhibition zones or bacterial growth around the discs.

After measuring the inhibition halos, the bacterial isolates were classified according to the Clinical and Laboratory Standards Institute CLSI (2018) as: Sensitive (S): the infection can be treated with the recommended dosage of the antimicrobial; Resistant (R): usual systemic concentrations of the antimicrobial do not inhibit the microorganism, generating clinical ineffectiveness; Intermediate (I): microorganisms

Table 1. Antibiotics used in antimicrobial susceptibility testing.

ANTIBIOTICS	AMOUNT ON DISCS
Amoxicillin + Clavulanic acid	30 µg
Ampicillin	10 µg
Azithromycin	15 µg
Cephalexin	30 µg
Cefoxitin	30 µg
Ciprofloxacin	5 µg
Clindamycin	2 µg
Erythromycin	15 µg
Gentamycin	10 µg
Penicillin	10 UI

with minimal inhibitory concentrations of the antimicrobial that reach systemic and tissue levels, but the response is low.

Figure 1 shows the schematic representation of the methodology for studying intramammary infections in goats in the city of Queimadas (Paraíba, Brazil).

RESULTS AND DISCUSSION

All the farms visited used a hand milking system, as observed by Peixoto *et al.* (2010) and Aragão *et al.* (2022) in their research conducted in northeastern Brazil. Conversely, Lima *et al.* (2018) found that milking was mechanised on 60% of the properties in the Zona da Mata region of Minas Gerais. These results align with those found by Farias *et al.* (2019), who identified a low level of technology in goat production systems in the Northeast, as well as inadequate or insufficient investment in technical assistance.

On eight farms, there was a milking platform present, with 25% of the structures made of masonry and 75% made of wood. However, two producers did not use the structure and milked their animals directly on the barn floor. These structures were built under the guidance of technicians from the agricultural department of Queimadas. Peixoto *et al.* (2012) and Machado *et al.* (2018) found a lower occurrence of mastitis on farms where milking was conducted on a platform compared to inside the barn, as the barn environment offers greater possibilities for contamination and complicates proper hygiene procedures.

It was noted that only two producers have a hand-washing sink near the milking area. All producers wash their hands with detergent before starting the process but do not carry out any sanitisation procedures between milking different animals. Aragão *et al.* (2022) found the presence of pathogens of various species on the hands of milkers, such as *Staphylococcus aureus* and *Staphylococcus haemolyticus*, which can jeopardise the health of both the animals and consumers of milk and dairy products. Similar results were reported by Silva *et al.* (2011), Monte *et al.* (2018), and Anderson *et al.* (2019), indicating that the milker's hands can be an important source of transmission of mastitis-causing microorganisms and milk contamination, highlighting the importance of good milking practices.

Regarding the evaluation of milk for alterations, all the producers discard the first three milk jets from each teat. This practice differs from findings by Silva *et al.* (2021), who reported that 94.8% of producers do not discard the first three milk jets in a study also conducted in Paraíba. In Queimadas, this practice is based on the guidance of technicians from the agriculture department. Only one producer reported carrying out the strip cup test, while another checks for clinical mastitis using an improvised instrument made from a PET bottle. The other producers do not perform any tests. Only one producer (12.5%) carries out pre-dipping with an applicator and commercial iodine-based products; the others clean the teats with chlorinated water and use paper towels to dry them. After milking, four producers reported post-dipping with an applicator and commercial iodine-based products, while the remaining four did not perform any post-milking hygiene process.

A study on dairy cattle by Santos *et al.* (2021) found a 94.33% reduction in Colony Forming Units (CFU) in the total bacterial count (TBC) between the first and third milk jets collected during milking, as well as a reduction of $8x10^4$ CFU/mL with the adoption of pre-dipping. Vallin *et al.* (2009) observed a reduction of more than 80% in the TBC of bovine milk after adopting a milking protocol that included discarding the first three milk jets and performing pre-dipping. Similarly, Ribeiro Júnior *et al.* (2014) found a reduction in the average TBC from $3.8x10^6$ CFU/mL to $1.8x10^5$ CFU/mL on farms that adopted a similar protocol. These results demonstrate the importance of

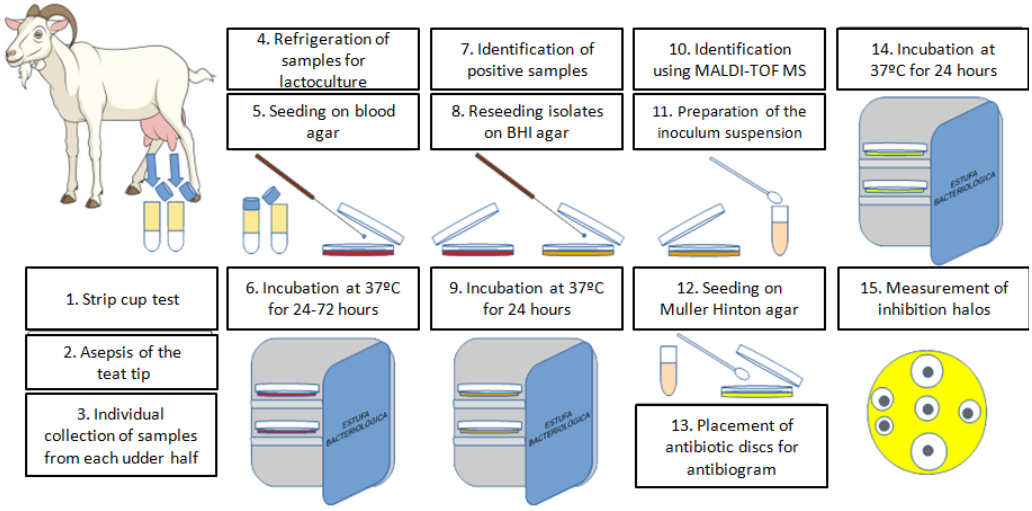

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Figure 1. Schematic representation of the methodology for studying intramammary infections in goats in the city of Queimadas (Paraíba, Brazil).

discarding the initial milk jets and properly sanitising the teats during milking.

The animals studied were mostly crossbred, with some identified as Saanen, Parda Alpina, and Toggenburg, similar to findings by Barros *et al.* (2018) and Riet-Correa *et al.* (2013). Regarding the stage of lactation, 21.05% were in the initial third, 1.75% were between 40 and 100 days of lactation, and 77.20% were over 100 days of lactation. Of the 57 goats studied, 19.3% were primiparous, 10.53% were in their second parity, 8.77% were in their third parity, and for the remaining 35 goats (61.4%), the producers were unable to provide precise information. Due to the recent start of organised production and marketing of goat's milk in Queimadas, most of the animals in the herds studied were purchased, and the farmers did not have precise information about many of them. This may reflect the low regional adherence to zootechnical bookkeeping, as also reported by Patês *et al.* (2012), Riet-Correa *et al.* (2013), Kato *et al.* (2019), and Helmer *et al.* (2020).

No clinical mastitis was found in any of the animals during milk sampling, a result that coincides with those of Schmidt *et al.* (2009) and Neves *et al.* (2010). However, producers mentioned a history of clinical mastitis in 10 goats, representing 17.54% of the animals studied, distributed over six of the eight participating herds.

To treat clinical mastitis, some farmers mentioned using parenteral medication with active substances such as tylosin, sulphadoxine and trimethoprim, as well as intramammary tubes suitable for cows' containing sulphadiazine and nystatin, amoxicillin and potassium clavulanate, cefoperazone sodium, ceftiofur hydrochloride, and ciprofloxacin. One farmer used garlic to treat a goat with clinical mastitis and did not use allopathic medication. Peixoto *et al.* (2013) noted that although it is common to use bovine medications to treat mammary infections in small ruminants, the causative agents and medication specifics, such as drug concentration, vary by species. Thus, this practice can lead to treatment failures in goats and sheep, resulting in animal welfare issues, as well as productive, economic, and health losses, including the emergence of antibiotic-resistant strains of microorganisms.

All producers reported deciding independently on which medication to use and how to conduct treatment, without consulting a veterinarian. Only one producer consulted a professional due to persistent symptoms in an animal. Langoni *et al.* (2017) emphasise that the use of antimicrobials requires care due to the disposal of residues in milk and the development of resistance in microorganisms. Therefore, Ruegg (2018) suggests that a qualified professional should choose treatment, preferably based on the farm's mastitis history and, where possible, lactoculture and antimicrobial sensitivity tests.

Bacterial growth was observed in 15 of the 113 udder halves, resulting in a subclinical mastitis prevalence of 13.27%. This result is close to those reported by Neves *et al.* (2010) (11.49%), Santos Júnior *et al.* (2019) (11.85%), Peixoto *et al.* (2012) (18.44%), and Gocmen *et al.* (2019) (12.37%, 19.58%, and 13.40% for the 10th, 20th, and 30th days postpartum, respectively), and lower than those found by Salaberry *et al.* (2015) (54.9%), Lima *et al.* (2018) (28%), and Machado *et al.* (2018) (34.7%). According to Contreras *et al.* (2007) and Peixoto, Mota, and Costa (2010), the prevalence of subclinical mastitis in small ruminants ranges from 5% to 30% and can be higher in specific cases, with a higher prevalence than the clinical form of the disease. Akter *et al.* (2020) suggest that differences in mastitis prevalence between herds are associated with various risk factors.

The positive samples came from 12 goats belonging to five herds. Bacteria identified included *Staphylococcus caprae*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Micrococcus luteus*, species also found by Langoni *et al.* (2012), Salaberry *et al.* (2015), Gosselin *et al.* (2018), Coimbra and Souza (2019), and Aragão *et al.* (2021).

These species can cause mastitis in goats, sheep, and cattle and are also important for human health. Coagulase-negative staphylococci, such as *Staphylococcus caprae*, *Staphylococcus epidermidis*, and *Staphylococcus lugdunensis* identified in this study are noted by Otto (2009), Gowda, Pensiero, and Packer (2018), and Heilbronner (2021) as natural colonisers of the skin and mucous membranes of humans, an ecological niche that, according to Ianniello *et al.* (2019), can also be occupied by *Micrococcus luteus*. According to Martín Guerra, Martín, and Rodriguez (2019) and Kosecka-Strojek *et al.* (2020), these are opportunistic pathogens that can colonise medical devices and materials, being particularly significant for orthopaedic patients (Seng *et al.*, 2014; Oliveira *et al.*, 2018; Widerström *et al.*, 2021), neonatal patients (Joubert *et al.*, 2022), and have also been reported to cause endocarditis (Seifert; Kaltheuner; Perdreau-Remington, 1995; Rodriguez-Nava *et al.*, 2020; Soca *et al.*, 2019; Hirose *et al.*, 2019) and nosocomial infections (Ogura *et al.*, 2022).

Figure 2 shows the results of the sensitivity tests of bacterial isolates of the species *Staphylococcus caprae*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* and *Micrococcus luteus* obtained from goat's milk in the city of Queimadas.

The bacterial isolates showed broad sensitivity to the 10 antibiotics tested, with a sensitivity profile higher than those found by Silva *et al.* (2004) (except for erythromycin), Peixoto *et al.* (2010), and Aragão *et al.* (2021), all in studies on small ruminant milk in northeastern Brazil. Only two isolates (25%) exhibited resistance: isolate 1 to azithromycin and erythromycin, and isolate 5 to azithromycin, both from animals with a history of clinical mastitis. Some antibiotics showed intermediate action on the isolates: erythromycin on isolates 2, 3, and 5, and ciprofloxacin on isolate 5.

According to Peixoto *et al.* (2010), the groups of antimicrobials used in human and veterinary medicine are often the same, leading to potential cross-resistance. The discovery of resistant strains, including one with multiple resistances, highlights the need for routine monitoring of the microbiological condition of milk from the herds studied. This is essential to

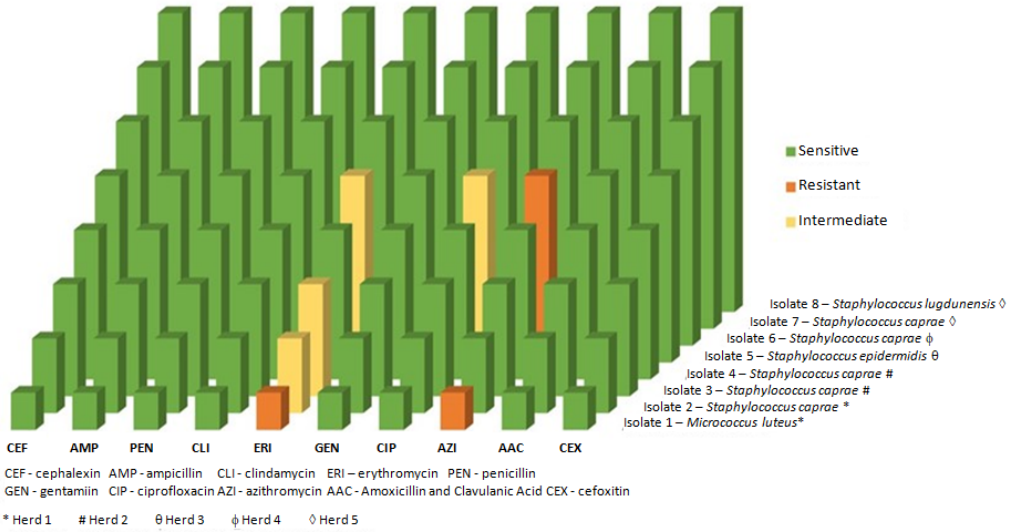

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Figure 2. Sensitivity of bacterial isolates obtained from goat's milk in Queimadas.

support educational and technical initiatives with producers in Queimadas regarding animal health management, ensuring food safety for producers and consumers and preventing the development of multi-resistant bacterial strains.

CONCLUSIONS

The dairy goat herds in Queimadas, Paraíba, Brazil, are managed with limited technological advancement. This is evident from the low adherence to proper milking hygiene practices and the incidence of clinical mastitis. Our study identified subclinical mastitis in these herds, caused by the bacteria *Staphylococcus caprae*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Micrococcus luteus*. Although the bacterial isolates demonstrated broad sensitivity to the antibiotics tested, some strains exhibited multiple antibiotic resistance. This underscores the necessity for routine microbiological monitoring and improved management practices to prevent the spread of resistant strains. Additionally, there is a crucial need for educational and technical support initiatives aimed at improving animal health management and ensuring food safety for both producers and consumers of goat milk in the region. These measures are essential for enhancing the overall productivity and sustainability of dairy goat farming in Queimadas.

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