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Detection of subclinical mastitis and characterization of *Staphylococcus* strains, in family-type goat herds

Detección de mastitis subclínica y caracterización de cepas de *Staphylococcus*, en rebaños caprinos de tipo familiar

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ABSTRACT

The objective was to identify subclinical mastitis using the California Mastitis Test (CMT) and to perform the phenotypic characterization of *Staphylococcus* strains. From February to May 2023, the study was conducted in 21 herds across six communities in the Santa Cruz de Juventino Rosas municipality, Guanajuato. Milk samples from 430 goats were analyzed using the CMT, and those testing positive were further sampled for bacteriological diagnosis. A total of 27.6 % of the goats tested positive for subclinical mastitis. The bacteriological analysis identified *Staphylococcus haemolyticus*, *S. hyicus*, *S. chromogenes*, *S. caprae*, and *S. epidermidis*, with resistance observed to cefotaxime, a third-generation cephalosporin. In conclusion, the study revealed a high prevalence of subclinical mastitis in the herds, with coagulase-negative *Staphylococcus* being the primary causative agent. Training programs for goat farmers are necessary to improve mastitis control through the adoption of good milking practices and diagnostic tools for prompt detection.

KEY WORDS: Goats, milking routine, mastitis, bacteriological diagnosis.

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RESUMEN

El objetivo fue identificar la mastitis subclínica mediante la prueba de California, y la caracterización fenotípica de cepas de *Staphylococcus*. De febrero a mayo de 2023 se trabajó en 21 rebaños de seis comunidades del municipio de Santa Cruz de Juventino Rosas, Guanajuato. Se analizaron por la prueba de California la leche de 430 cabras, de las positivas a la prueba se colectó leche para diagnóstico bacteriológico. El 27.6 % de las cabras fueron positivas a la prueba de California. En el estudio bacteriológico, se identificaron a *Staphylococcus haemolyticus*, *S. hyicus*, S. *chromogenes*, *S. caprae* y *S. epidermidis,* se observó resistencia a la cefotaxima, una cefalosporina de tercera generación. En conclusión, el estudio mostró que la mayoría de las cabras se vieron afectadas por mastitis subclínica, siendo la principal bacteria causante *Staphylococcus c*oagulasa negativo. Es necesario capacitar a los caprinocultores para mejorar el control de la mastitis mediante la adopción de buenas prácticas de ordeño y el uso del diagnóstico para la detección de la mastitis.

PALABRAS CLAVE: Caprinos, ordeña, mastitis, diagnóstico bacteriológico.

Introduction

Goat farming contributes 2 % of global milk production (FAO, 2024). Mexican goat farmers account for 0.9 % of the global volume, ranking among 112 countries dedicated to goat milk production. In Mexico, 169 million liters of goat milk were produced in 2023, representing 0.7 % of the national livestock production. The leading goat milk-producing states include Coahuila (northeastern region), which contributes 27 % of national production, followed by Guanajuato (central-western region) with 26.8 %, and Durango (northeastern region) with 14.6 %. Other states, such as Jalisco, Chihuahua, Zacatecas, San Luis Potosí, Nuevo León, Baja California Sur, and Michoacán, contribute between 2.5 % and 4.6 %, while the remaining states have a lower share (SIAP, 2024).

Goat farming is a traditional practice, primarily conducted as a family-based activity. Most production units consist of small herds managed directly by a shepherd or family, who handle all management tasks (Guerrero, 2010).

Mastitis is one of the most significant diseases in the dairy industry, causing substantial economic losses in production units. The main predisposing factors include poor sanitary

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conditions within the herd, lack of therapeutic and control measures, and inadequate hygiene during and after milking (Abdelrahman *et al.,* 2020).

The clinical mastitis incidence in goats is generally below 5 %; however, the estimated prevalence of subclinical mastitis in small ruminants ranges from 5 % to 30 % (Contreras *et al.,* 2007). In Mexico, reported prevalence rates range from 20 % to 58 % (Ávalos-Castro *et al.,* 2022). To establish an effective control and treatment program, opportune detection of cases and the identification and characterization of causative agents are essential, subclinical mastitis presents no visible signs of disease, typically does not cause apparent changes in glandular tissue, and the milk appears normal. This mastitis type can only be detected by measuring the somatic cell content in milk (Ruiz, 1989; Shearer & Harris, 2003). One of the most commonly used methods in production units for this purpose is the California Mastitis Test (CMT); however, identifying the etiological agents involved requires bacterial isolation (Kabui, 2024).

In goats, coagulase-negative *Staphylococcus* (CNS) is the primary causative agents of subclinical mastitis. These bacteria can develop resistance to common antimicrobial treatments, making timely field detection and laboratory confirmation essential for establishing effective treatments. Additionally, reinforcing good milking practices can help reduce their spread (Contreras *et al.,* 2007). Therefore, this study aimed to identify subclinical mastitis using the California Mastitis Test, determine the *Staphylococcus* species involved in its occurrence, and assess their potential antimicrobial resistance in family-managed goat herds.

Material and Methods

The study was conducted from February to May 2023 in family-managed goat herds from six communities in the Santa Cruz de Juventino Rosas municipality, Guanajuato, Mexico (Table 1). A total of 21 voluntary producers participated, all located in areas with low to very low marginalization indices. The selected herds had a maximum of 70 goats, with backyard agriculture and/or livestock as their primary source of income. Preference was given to producers utilizing local resources such as rangelands or communal lands for grazing and those with limited or no technical assistance.

Each herd underwent a clinical inspection of the mammary glands in lactating goats. The California Mastitis Test (CMT) was performed on all animals (Hernández & Bedolla, 2008); approximately 3 mL of milk from each half of the udder was collected and placed in separate wells of a CMT paddle. An equal amount of CMT reagent (Nuplen Químicos, Mexico) was then added, and the mixture was gently swirled for approximately 30 seconds. Samples showing a gel-like reaction were considered positive, while those without visible changes were deemed negative (Hernández & Bedolla, 2008). From all CMT-positive goats, 30 mL of milk were collected in sterile, labeled 50 mL tubes for bacteriological analysis. The samples were transported on ice to the Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, stored at 4°C, and processed within 8 hours of collection.

For *Staphylococcus* identification, milk samples were brought to room temperature, homogenized, and inoculated on blood agar (Mačešić et al., 2022). The plates were incubated under aerobic conditions at 37 °C for 48 hours. Suspect colonies were macroscopically characterized based on colonial morphology, followed by Gram staining and microscopic examination for bacterial morphology. Biochemical tests, including catalase, oxidase, and coagulase assays, were performed to confirm identification. Species determination was carried out using the API Staph® commercial identification system (Biomérieux Laboratories, France).

Antibiotic susceptibility testing was performed using the disk diffusion method (Pum, 2019). *Staphylococcus* isolates were standardized using a turbidity test (0.5 McFarland standard) before inoculating 100 µL of the standardized suspension onto Müller-Hinton agar plates (BD, South Africa). The following antibiotic-impregnated disks (Bio-Rad Laboratories, USA) were used: amikacin (30 µg), ampicillin (10 µg), levofloxacin (5 µg), cephalothin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), netilmicin (30 µg), nitrofurantoin (300 µg), cefepime (30 µg), and trimethoprim-sulfamethoxazole (25 µg), the disks were placed on the agar surface, and the plates were incubated at 37 °C for 24 hours. Inhibition halos were measured, and results were interpreted according to CLSI guidelines (Pum, 2019).

The results were integrated into a database for analysis and determination of frequencies of subclinical mastitis in each community and herd. Homogeneity tests of proportions were performed by Chi-square (X^2) according to the milking type, manual and mechanical, with respect to the isolates obtained and the detection of subclinical mastitis with the CMT test. The value of p <0.05 was considered statistically significant. Statistical analyses were performed using the commercial statistical software SPSS version 25.0 (SPSS Inc., USA).

Results and Discussion

Of the 430 lactating goats evaluated in this study, 27.7 % tested positive for CMT. Bacteriological analysis identified *Staphylococcus* isolates in 119 CMT-positive milk samples, with a total of eleven *Staphylococcus* isolates classified as follows: *S. haemolyticus* (n=1), *S. hyicus* (n=1), *S. chromogenes* (n=1), *S. caprae* (n=2), and *S. epidermidis* (n=6) (Table 1).

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Table 1. *Staphylococcus* species identified by community.

	Community	PU[1]	Positive animals	*Staphylococcus* species identified
1	Municipality's capital	6	24	*S. haemolyticus* and *S. hyicus*
2	Emiliano Zapata	2	44	*S. epidermidis*
3	Cerrito de Gasca	2	5	No bacterial isolate
4	Naranjillo	5	16	*S. epidermidis*
5	San Juan de la Cruz	4	28	*S. caprae* and *S. chromogenes*
6	La Purísima	2	2	No bacterial isolate
	Total samples	21	119	

Production units/Farm.

Most of the isolates obtained in this study correspond to coagulase-negative *Staphylococcus* (CNS), except for *S. hyicus*, similar to findings reported by Ávalos-Castro *et al.* (2022). In goats, CNS species are the most frequently detected bacteria in subclinical infections, with detection rates ranging from 25 % to 93 %, consistent with our findings (Donmez & Kirkan, 2022).

Although these species are less pathogenic than *S. aureus*, they can cause persistent infections lasting for months, including the dry period, significantly increasing somatic cell counts and leading to clinical mastitis (Bedolla *et al.,* 2012). While no goats were identified with clinical mastitis during CMT testing in this study, producers reported a higher incidence of clinical cases during the rainy or winter seasons.

Among the main *Staphylococcus* species causing mastitis in goats are *S. epidermidis, S. caprae, S. simulans,* and *S. chromogenes*, all of which are associated with persistent infection, increased somatic cell counts, and reduced milk production (Virdis *et al.,* 2010; Bedolla *et al.,* 2012). In this research, *S. epidermidis* and *S. caprae* were the most frequently isolated species. However, even though only a single isolate was found for some *Staphylococcus* species, these bacteria are opportunistic and can thrive in a wide range of ecological habitats, including udder skin, teat canals, bedding, milk tanks, and feces, which explains their persistence in herds (Piessens *et al.,* 2011; Jesse *et al.,* 2023). *S. chromogenes* and *S. haemolyticus* can colonize the skin, udder, and distal portion of the teat canal (Braem *et al.,* 2013; Bexiga *et al.,* 2014).

Poor cleaning and disinfection of milking units, along with a lack of proper hygiene practices during milking, are considered the primary transmission routes for mastitis, leading to reduced milk production and overall herd productivity (Contreras *et al.,* 2007; De Visscher *et al.,* 2014).

Observations from the PUs in this study revealed that 80.9 % of the units practiced manual milking within pens, often in non-designated areas and without adequate hygiene measures. Although four PUs used mechanical milking and implemented hygiene practices such as teat cleaning before milking, drying with individual disposable materials, forestripping, and teat sealing, there was no statistically significant difference in bacterial isolation between mechanical and manual milking methods (X^2 = 0.35, p = 0.51). This suggests that proper milking hygiene practices were not consistently applied in either method. However, subclinical mastitis detection was higher in manually milked goats (41.1 %) compared to those milked mechanically (38.6 %) (X^2 = 88, p = 0.001), aligning with findings by Ávalos-Castro *et al.* (2022). Some studies indicate that implementing proper hygiene practices can effectively reduce the incidence of subclinical mastitis (Smith *et al.,* 2015).

Mastitis is a multifactorial disease, with non-infectious causes such as udder contusions from fights, lacerations, and cutaneous myiasis (Ordoñez *et al.,* 2022). Additionally, other infectious agents, including bacterial, fungal, or viral pathogens, can predispose goats to mastitis (Menzies, 2018; Ordoñez *et al.,* 2022). This could explain the discrepancy between bacterial growth results and the total number of CMT-positive samples.

Hence, several factors may be related to the increase in somatic cells in milk, which are indirectly detected by the CMT test through the reaction of bromocresol purple with the genetic material of the cells present in the milk (Maisi & Riipinen, 1988). A negative result in this test is a good indicator of the absence of infection, while a positive result does not always indicate an infectious process in the mammary gland. This is due to the higher presence of epithelial cells compared to cow's milk, as in goats; these cells are a natural component of protection (Li *et al.,* 2014; Machado, 2018).

Gelasakis *et al.* (2016) question the usefulness of the CMT test as a precise diagnostic tool for subclinical mastitis in goats, as the increase in somatic cells in milk may also be associated with physiological factors such as the number of births, lactation stage, estrus, stress, and even breed. This can lead to the analysis of milk samples that are more likely to be negative in bacteriological culture. Despite this, other studies emphasize the importance of the test as a useful indicator for goat farmers in identifying deficiencies in milking hygiene management; also, it is a low-cost test that is easy to apply in the field and should be accompanied by bacteriological diagnosis (Bazan *et al.,* 2009; Machado, 2018; Mahlangu *et al.,* 2018).

From a productive perspective, this disease reduces herd productivity due to lower milk production, alterations in the offspring weight, and, consequently, decreased availability for transformation into cheese and sweets, as well as for direct consumption (Contreras *et al.,* 2007; Giboin *et al.,* 2019). In France, milk production losses are estimated to range from 55 to 132 kg per year, with a reduction of 0.3 g of fat per kg of milk per animal (Baudry *et al.,* 1997).

Antimicrobial resistance in bacteria is an increasing global concern (Chokshi *et al.,* 2019). This phenomenon has been observed in CNS, showing resistance to first-, second-, and third-generation cephalosporins. This occurs because these antibiotics are frequently used to treat

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mastitis and other infectious diseases due to their efficacy against most Gram-positive bacteria, including streptococci and staphylococci (Chavarría & Meléndez, 2012; Pascu *et al.,* 2022). In this study, none of the isolated *Staphylococcus* species were resistant to cephalothin. However, all isolates were sensitive to netilmicin and gentamicin. Although it was not possible to determine the type or origin of CNS resistance in this study, some research suggests that increasing antimicrobial resistance may be related to the genetic variability of isolates, climatic differences, and geographical discrepancies, among other factors (Karzis *et al.,* 2019).

It is important to highlight that the herds considered in this study do not receive technical assistance from a veterinarian. In Mexico, this is common in family-run production units, where, when animals develop diseases, producers often consult other farmers with similar experiences or seek advice at veterinary pharmacies. In these cases, treatments are recommended based on the producers' descriptions, which can contribute to the emergence of resistant bacterial strains due to improper treatment administration.

Conclusions

Based on the results obtained in this study, we consider that the CMT is a suitable tool for detecting the presence of somatic cells in goat milk; however, it is not sufficient to individually diagnose subclinical mastitis in goats. Therefore, CMT results indicating an increase in somatic cells should be considered to review potential deficiencies in milking best practices. To address these issues, a traceability analysis should be conducted to identify critical points in the process where failures occur. Modifications should be made to conditions that may contribute to an increase in somatic cells and the occurrence of subclinical mastitis. We recommend bacteriological isolation and antibiotic susceptibility testing for the latter to minimize the risk of developing resistant bacterial strains.

Author contributions

Work conceptualization: GHJL, PREG; Methodology development: ARSL, TLC, SGDC, SRR; Software management: ARSL, TLC; Experimental validation: GHJL, PREG; Data analysis: ARSL, PREG, TLC, SGDC; Data management: ARSL, TLC, AGS; Manuscript writing and preparation: PREG, AGS, DAE; Writing, review, and editing: All authors; Project administration: GHJL; Funding acquisition: GHJL

"All authors have read and approved the published version of this manuscript."

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Conflict of interest

The authors declare no conflict of interest.

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