Determination of risk factors related to prevalence of subclinical mastitis in dairy goats in Boyacá- Colombia

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ABSTRACT

Objective. Determine the prevalence of subclinical mastitis and the main risk factors associated with its presentation in the goat production systems of the municipality of Boavita. **Materials and methods.** A cross-sectional study was carried out between the months of March to July 2021 in 15 farms in the municipality. 256 lactating goats without the presence of clinical mastitis in any of the udder halves were included, a milk sample was taken prior to milking, and it was transported in refrigeration until arrival at the laboratory for evaluation through CMT. The risk factors determined were subjected to univariate and multivariate statistical analysis using the Statgraphics Centurion X statistical program. **Results.** The general prevalence of subclinical mastitis was 26.56% and the main associated risk factors were: more than three years, number of births greater than five, days in milk production, absence of disinfection processes prior to milking, poor body condition and presence of lesions on the nipples. **Conclusions**. For the first time in the country, the prevalence and the main risk factors associated with it were determined in dairy goat herds located in the municipality of Boavita, Boyacá.

Keywords: Mammary gland; inflammation; lactation; risk; ruminant (*Source: MeSH*).

RESUMEN

Objetivo. Determinar la prevalencia de mastitis subclínica y los principales factores de riesgo asociados a su presentación en los sistemas productivos caprinos del municipio de Boavita. **Materiales y métodos.** Se realizó un estudio de tipo transversal entre los meses de marzo a julio de 2021 en 15 granjas del municipio. Se incluyeron 256 cabras lactantes sin presencia de mastitis clínica en alguna de las mitades de la ubre, se les tomó una muestra de leche previo al ordeño y fue transportada en refrigeración hasta su arribo al laboratorio para la evaluación a través de CMT. Los factores de riesgo determinados fueron sometidos a análisis estadístico univariado y multivariado a través del programa estadístico Statgraphics Centurion X. **Resultados.** La prevalencia general

de mastitis subclínica fue de 26.56% y los principales factores de riesgo asociados, con un valor p <0.05, fueron: más de tres años, número de partos superior a cinco, días en producción de leche, ausencia de procesos de desinfección previo al ordeño, pobre condición corporal y presencia de lesiones en los pezones. **Conclusiones.** Se determinó, por primera vez en el país, la prevalencia y los principales factores de riesgo asociados con esta en rebaños lecheros caprinos ubicados en el municipio de Boavita, Boyacá.

Palabras clave: Glándula mamaria; inflamación; lactancia; riesgo; rumiante (*Fuente: MeSH*).

INTRODUCTION

Goat milk consumption has increased in industrialized and developing countries. This behavior has been associated mainly with three fundamental factors. The first of these is the accessibility that this product has in homes for feeding people in a state of vulnerability. The second is the advantage that it presents compared to bovine milk in the processes of transformation into dairy products such as cheese and yogurt, and the third is derived from medical purposes, mainly in the pediatric and geriatric population, as well as in those people who have allergies to cow's milk and other eating disorders, including people with respiratory diseases such as asthma, colds, and eczema (1,2,3,4)

However, the nutritional quality of raw milk depends on factors such as correct feeding and handling, the adequate health status of the mammary gland, and the correct storage of milk after milking. The productivity of dairy goat companies is widely affected by multiple factors, one of the most important is the presence of mastitis in any of its forms (clinical, subclinical, and chronic mastitis) (5,6).

Subclinical mastitis is a common disease in herds worldwide and is perhaps the factor that most affects milk quality (7,8). This is caused by infection of the mammary gland by microorganisms such as bacteria and fungi, either derived from the environment (or environmental mastitis) or transmitted by other animals (infectious mastitis) (8,9,10). Regardless of their name, the microorganisms involved enter through the teat canal and colonize the entire glandular structure causing inflammation, which leads to an increase in somatic cell count and enzymatic processes that alter milk. Its clinical diagnosis is complex because in this form there are no apparent changes in the mammary gland such as redness, pain, or inflammation, however, production decreases by up to 45% in quantity and 40% in quality (6,8,11).

Contrary to clinical mastitis, which is detected through clinical examination, subclinical mastitis does not present apparent clinical manifestations, therefore, its detection must be carried out through various tests such as microbiological diagnosis, somatic cell count, or California mastitis test. The latter uses a reagent that meets the somatic cells present and allows visualizing and classifying the physical change that the milk undergoes, relating it to the presence or not of the disease to varying degrees (6,12,13,14).

Boyacá has been characterized by its goat production distributed mainly in the municipalities belonging to the provinces of Norte and Gutiérrez, within these, the municipality of Boavita has a population of goats estimated at 3209 individuals, demonstrating its importance for the local peasant family economy (15). The main productive focus in the region is meat production, however, the producers have associated themselves and have derived around 1000 animals to dairy production with the subsequent transformation to products such as cheese and yogurt, however, the lack of constant advice that covers all aspects related to animal health and, in general, the production system, has prevented milk production and its quality from growing gradually, because at this time the average production per animal is around 350 milliliters in a daily milking (15).

In these goat productions, there is no program for the detection, prevention, and control of mammary gland diseases that is standardized and allows producers to identify mastitis early, ensuring product quality, improving marketing possibilities, and thus increasing productivity and quality of life of producers. Therefore, the first step is to identify those external factors

that are related to the presence of subclinical mastitis within the herds. Therefore, the objective of this study is to identify the risk factors associated with the prevalence of subclinical mastitis in goat herds located in Boavita-Boyacá in Colombia.

MATERIALS AND METHODS

Type of study. A cross-sectional study was carried out between March to July 2021 in 25 farms in the municipality of Boavita in the north of the department of Boyacá to determine the prevalence of subclinical mastitis in the herds, the samplings were carried out during this period on different farms only once in each visit. The municipality of Boavita has an area of 146 square kilometers, is located at a height of 2150 meters above sea level, its average temperature is 17°C and rainfall is 1,112 millimeters. It is located 187 kilometers (Km) from Tunja, (capital of the department of Boyacá) and 260 km from Bogotá D.C (capital of Colombia) (16).

Farms. Producers who expressed interest in participating in the study were included. No excluding factor was considered for farms. A convenience classification was made according to the number of goats present in the production system in the following way: small farms with less than 13 goats in production; large farms with more than 14 goats in production. The maximum size of the farms was 25 milking goats. In total, 25 farms distributed throughout the municipality were included. In all production systems, milking was done manually once a day, between 5:00 a.m. and 6:30 a.m.

Type of accommodation. Two types of accommodation were identified, in the first, called "grazing", the animals remained in the paddock throughout the day and were only picked up at night to remain in the stable, where they were milked in the morning before leaving new to herding. In the second, the goats remained in constant housing.

Animals included in the study. 256 lactating goats were included, with different parity numbers and lactation statuses, which were distributed in the different production systems. The goats were aged between 18 months and 84 months, with an average value of 27 months. Goats showing manifestations of clinical mastitis (swelling, pain, redness, and heat in case of acute mastitis and hardening of the udder, teat plugging, atrophy, or fibrosis in case of chronic mastitis) were excluded (17). Likewise, samples were only taken from goats with days in milk (DIM) greater than three and up to 120 days in production. No purebred animals were found on the farms, however, crosses between Saanen, Anglonubian, and Toggenburg breeds were found.

Sampling. Before collecting the milk sample, adequate cleaning and dryness of the teats were ensured. Each teat was cleaned with a cotton pad moistened with 70% alcohol. The first milk ejection was discarded, and individual samples were collected from each half of the mammary gland. Approximately 20 mL of each glandular half was collected in sterile plastic containers. Immediately after collection, milk samples were subjected to physical examination with the naked eye to detect any abnormalities in color, odor, consistency, presence of blood and clots, and any other visible abnormalities (3,7,8).

Each sample was labeled with an identifier for the animal given by the owner, and a number for the farm given by the researchers. Likewise, as the sample was taken, relevant information about the animal and the farm was recorded. Each animal was sampled only once during the study. Sampling was carried out every 15 days, for two consecutive months.

California Mastitis Test and Interpretation. After collection, each milk sample was packed in ice and kept at a temperature between 4 and 6°C until it arrived at the milk quality analysis and mastitis control laboratory of the Pedagogical and Technological University of Colombia, where the California Mastitis Test (CMT) was performed 5 hours after sampling. This was done by mixing 3 mL of reagent (alkyl aryl sulfonate) with 3 ml of the milk sample on the CMT plastic paddle, then homogenizing them with a circular motion for approximately 20 seconds (8,9,10,12).

The results were scored from 0 to 5 depending on the degree of gel formation. The scores depend on the degree of gelation indicated by the gelatinous mass in proportion to the severity of the infection present (18). The interpretation of the results was as follows: CMT 0 and CMT 1: negative. CMT 2: positive for subclinical mastitis. CMT 3 to 5: clinical mastitis (12).

Registered information of each farm. The information that was recorded was: the total number of goats in production, age, number of births, days in milk (DIM), amount of milk yield expressed in liters/day, and previous history of mastitis in individual animals, this information was taken with the help of the farmer. Likewise, the researchers determined, without the help of the owner: the routine of disinfection of the teat before milking, type of accommodation, and body condition based on what was reported by Ghosh et al (19) and Vieira et al (20), in an analysis of five scales is made for the evaluation of body condition, however, for this research, body conditions 1 and 2 will be reported as "poor" while body conditions 3 and 4 will be "good". Teat lesions and teat size (8).

Statistical analysis. The information collected was recorded directly in each farm in the Microsoft Excel program, later, to carry out the statistical analyzes, the Statgraphics Centurion X program for Windows 8.1 was used. Univariate analysis was performed to determine the association of individual variables with mastitis prevalence using logistic regression analysis. Those variables that did not show a statistically significant relationship (p>0.05) with the prevalence of subclinical mastitis through this analysis were discarded for the following statistical model. Multivariate logistic regression analysis was performed to identify those factors that contribute to the prevalence of subclinical mastitis in goats, the odds ratio was used to determine the strength of the associations identified in logistic regression. The value was considered significant when p<0.05 (6,21).

RESULTS

Univariate Analysis. The general prevalence determined in this study (considering only those results of CMT 2) was 26.56%. The results of the univariate analysis are presented in Table 1. In terms of age and number of deliveries, the highest prevalence was found in the groups older than 4 years and 6 deliveries, respectively, giving rise to a greater probability of manifestation of subclinical mastitis compared with animals between one and two years and between one and two births (p<0.05). A higher probability of the presence of the disease was found in goats with more than 60 days in production. It was also statistically determined that there is a much higher probability of presenting subclinical mastitis in those animals that did not undergo teat disinfection before milking, poor body condition, and teat lesions. Factors such as milk production, herd size, housing type, as well as teat size did not show a statistically significant association (p>0.05) with the presence of subclinical mastitis in dairy herds (Table 1).

Multivariate analysis. For the multivariate regression analysis, those variables that did not show a statistical relationship through a univariate analysis were excluded. In this sense, the following parameters were eliminated from this analysis: milk yield, herd size, type of housing, and teat size. The statistical analysis shows that variables associated with the animal such as age (>4 years), the number of births (>6), and more than 90 days in production, as well as variables associated with management within the farm such as teat disinfection, body condition and the presence of teat lesions, have a strong statistical association, even though these data are similar to those found in the univariate analysis, it is evident that the odds ratio and p-values for this multivariate analysis are much lower than in the first (Table 2).

Table 1. Univariate analysis of the potential risk factors that are hypothesized to be associated with the presentation of subclinical mastitis in dairy goats in the municipality of Boavita.

Risk factors	Levels	Number evaluated	Positive number s	Prevalence (%)	Odds ratio	CI* (95%)	P-value (<0.05)
Age	1-2 years	89	12	13.48	1		
	2-3 years	60	20	33.33	1.3	0.6/2.3	0.745
	3-4 years	56	27	48.21	1.7	0.8/3.3	0.048
	>4 years	51	26	50.98	2.2	1.1/3.9	0.024
Calving	1-2	55	8	14.54	1		
	3-4	93	25	26.88	1.25	0.6/1.8	0.810
	5-6	53	18	33.96	2.32	1.1/4.6	0.038
	>6	55	25	45.55	2.5	1.2/4.7	0.029
DIM	3 – 30	34	13	38.23	1		
	31 – 60	79	18	22.78	1.2	0.9/2.3	0.715
	61 – 90	115	45	39.13	1.8	0.7/3.6	0.060
	91 – 120	28	19	67.85	4.3	2.1/8.4	0.020
Milk production (liters-lt)	< 1 lt	78	32	41.02	1		
	1 – 2 lts	142	46	32.39	1.26	0.4/2.2	0.654
	>2 lts	36	12	33.33	1.35	0.6/2.6	0.0587
Herd size	<13	165	68	41.21	1		
	>14	91	23	25.27	0.6	0.3/1.2	0.157
Type of accommodation	Grazing	210	67	31.90	1		
	Confinement	45	12	26.66	1.2	0.5/1.8	0.410
Teat disinfection	Yes	215	45	20.93	1		
	No	41	35	85.36	4.3	2.3/8.4	0.010
Body condition	Good	100	15	15	1		
	Poor	156	60	38.46	2.2	1.3/3.9	0.032
Teat injuries	No	200	30	15	1		
	Yes	56	40	71.42	4.2	2.3/5.8	0.016
Teat size	< 2 cm	117	25	21.36	1		
	> 2 cm	139	38	27.33	1.2	0.8/2.1	0.063

*CI: confidence interval.

Table 2. Multivariate logistic regression analysis of the potential risk factors associated with the prevalence of subclinical mastitis in dairy goats in the municipality of Boavita.

Risk Factor	Levels	Number	Positives %	Odds ratio	CI (95%)	P-value
Age	1-2 years	89	13.48%	1		
	2-3 years	60	33.33%	1.1	0.4/1.9	0.873
	3-4 years	56	48.21%	1.9	0.8/3.9	0.045
	>4 years	51	50.98%	2.6	1.3/5.1	0.024
Calving	1-2	55	14.54%	1		
	3-4	93	26.88%	1.35	0.8/2.4	0.654
	5-6	53	33.96%	2.76	1.7/5.4	0.022
	>6	55	45.55%	3	1.7/5.9	0.012
DIM	3 – 30	13	38.23%	1		
	31 – 60	18	22.78%	1.1	0.5/1.6	0.834
	61 – 90	45	39.13%	2.1	1.2/4.4	0.042
	91 – 120	19	67.85%	4.7	2.5/9.7	0.010
Teat disinfection	Yes	215	20.93%	1		
	No	41	85.36%	5.4	2.8/10.6	0.012
Body condition	Good	100	15%	1		
	Poor	156	42.94%	3.2	1.8/5.8	0.010
Teat lesions	No	200	15%	1		
	Yes	56	71.42%	4.6	2.6/5.3	0.013

DISCUSSION

This is the first study carried out in Colombia where the prevalence of subclinical mastitis is analyzed, as well as its association with the main risk factors in dairy goat farms. The general prevalence of subclinical mastitis in the present study was 26.56%, which is consistent with that reported by Contreras et al (11), where the presence of the disease in herds can range between 5 and 30%, as is the case in Ethiopia, some authors have reported a prevalence of 11.2% and 38.6% (6,22), in that order of ideas, the results found in the evaluated properties would be in the estimated range.

However, all these results are lower than those found by Mahlangu et al (18) who determined a general prevalence of 40.5% in goat herds located in Kenya. The discrepancy between the results may be linked mainly to zootechnical factors such as the management of the milking routine because in this study the animals were milked only once, and the breed of the animals due to the crosses present could predispose some more animals to mastitis, the diagnostic methods used, as well as the geographical characteristics of the Boavita area. For this reason, studies of this type must be carried out throughout the production systems in each country and in each region, to obtain contextualized data on the situation in each area.

Regarding the main associated risk factors, previous studies have shown that a late state of milk production (>90 days), number of births greater than 3, poor body condition (on the scale used conditions 1 and 2), size of teats >2 centimeters and the absence of pre-milking teat cleaning programs are associated with an increased prevalence of subclinical mastitis in dairy herds (6,12,13,14,17,18,22).

The results found in this study are in agreement with those obtained by the previously mentioned investigations. These results may be because the region where this study was carried out is a semi-arid zone, with a height above sea level above 1800 meters, with a sloping topography with soil characteristics with a high rock content. In addition to this, there is the presence of plants from families such as the *Cactaceae*, and shrubs such as the species *Pithecellobium dulce* and *Swinglea glutinosa* (16). All this makes the animals that graze on these slopes susceptible to suffering processes of damage to their udders.

Therefore, there could be similarities concerning the behavior of the animals used in the studies and, therefore, concordances can be found in the results of risk factors, especially in other studies that evaluate animals from arid or semi-arid regions like those found at Boavita (6,12,13,14,17,18,22).

In Mexico, a prevalence of general subclinical mastitis of 30.5% has been determined in the evaluated dairy herds, associating it mainly with herds with quantities of less than 51 animals and with herds above 151 animals, that is, small and large herds, respectively. as well as in herds with intensive productive management (23). In this study, no herd had sizes greater than 200 animals, likewise, the type of production system is semi-intensive, which could explain the lower prevalence in this study compared to the Mexican one, likewise, a statistically significant relationship was not found either difference between herd size and mastitis prevalence.

In Venezuela, even when they did not associate risk factors, they determined a prevalence of 28% of milk samples in which there was the presence of bacterial growth associated with subclinical mastitis, which is a value like that found in this study (24).

Other risk factors associated with the presentation of subclinical mastitis measured through the somatic cell count by the CMT, and that were not evaluated in this research are breed, seasonal variations, types of feeding, and milking methods (25,26). It has also been shown that a simultaneous study of subclinical mastitis analysis and pathogen detection through laboratory isolates improves the understanding of the dynamics of the disease within herds (25,27). This was done in this study, however, only the results related to risk factors are presented in this paper.

It has been widely documented how the presence of subclinical mastitis is associated with a detriment in the nutritional quality of milk (7,8,9). This loss in quality is related to inflammatory processes in the udder, which alter the passage of nutrients from the blood and, in addition, to enzymatic degradation processes due to the increase in the somatic

cell count (28). Another important factor to take into account is the presence of pathogenic microorganisms which could trigger these inflammatory processes (but they are not the only ones, because there may be aseptic factors that trigger inflammation), in addition, these microorganisms use milk as a means to favor its growth, generating an increase in milk degradation (12,13,14,17).

The purpose of the milk produced by the goats of the farmers who participated in the study is its transformation into products such as cheese and yogurt, therefore, it is important to evaluate the presence of this disease in animals because, as already stated, their presence can affect the quality of the processed product. This is because the milk must have the capacity to tolerate the technological treatments that it must undergo to be transformed and thus meet the expectations of the consumers (5). Therefore, if farmers improve their production processes by analyzing the risk factors that were determined, they can improve their purchase options and thus increase their income, improving their quality of life (7).

In conclusions, it was determined that the main risk factors associated with the prevalence of subclinical mastitis in dairy goats in the municipality of Boavita in the department of Boyacá are: >3 years, several births greater than five, days in milk production, absence of processes of disinfection before milking, poor body condition, and presence of teat lesion.

Conflict of interests

The authors declare that there are no conflicts of interest during the planning, implementation, writing, and presentation of the document to the Journal MVZ Córdoba.

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