Mastitis in dairy goats from the state of Minas Gerais, Brazil: profiles of farms, risk factors and characterization of bacteria[1]

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ABSTRACT.- Lima M.C., Souza M.C.C., Espeschit I.F., Maciel P.A.C.C., Sousa J.E., Moraes G.F., Ribeiro Filho J.D. & Moreira M.A.S. 2018. **Mastitis in dairy goats from the state of Minas Gerais, Brazil: profiles of farms, risk factors and characterization of bacteria.** *Pesquisa Veterinária Brasilera 38(9):1742-1751.* Laboratório de Doenças Bacterianas, Departamento de Veterinária, Universidade Federal de Viçosa, Avenida PH Rolfs s/n, Centro, Viçosa, MG 36570-900, Brazil. E-mail: masm@ufv.br

The Zona da Mata of Minas Gerais has a specialized goat milk production chain. Goat milk is superior in quality compared with milk of other domestic species, and the demand for milk and milk products for the public has increased. Data on dairy goat breeding in Minas Gerais are scarce and relatively old, and this lack of information has limited the implementation of prophylactic measures, especially for mastitis, which represents the biggest sanitary problem for dairy herds. The objective of this work was to characterize mastitis and bacteria associated with it in milking goats in the Zona da Mata of Minas Gerais. It also causes socioeconomic problems and market issues for dairy goat farming. A total of 539 lactating goats were examined and 268 individual samples (one for teat) were collected from animals positive for strip cup test and/or the California Mastitis Test (CMT). Microbiological cultures were carried out on blood agar medium and the bacteria were subjected to phenotypic, genotypic and antimicrobial susceptibility tests. The prevalence of subclinical mastitis was 28.0% and the clinical prevalence was 2.8%. Bacterial multiplication was obtained in 62% of samples. One hundred eighty seven total bacteria were identified. The most common species identified was *Staphylococcus aureus* (60%), followed *Staphylococcus epidermidis* (9.1%,), *Escherichia coli* (6.9%), *Staphylococcus saprophyticus* (5.9%) e *Staphylococcus caprae* (4.3%). Bacteria of the genus *Staphylococcus* presented a profile of resistance to antimicrobials belonging to the beta-lactam class (penicillin, ampicillin and oxacillin) in addition to tetracycline, in contrast to the other antimicrobials tested. Twelve percent of multidrug resistence (MDR) was found in five microregions. Among the bacteria with the highest prevalence of MDR, 38.5% were *E. coli* and 10.6% were *S. aureus*. The producers of the Zona da Mata of Minas Gerais are technicians who work with specialized dairy breeds and practise good management. However, some measures related to prophylaxis and control of diseases, such as vaccination, have low adherence or are not performed due to a lack of veterinary assistance. This is the first study focusing on this region, which is highly prominent in goat milk production in Brazil. It provides important information that can help in the implementation of measures for the prophylaxis and control of diseases, and for maintenance of a constant supply of products in sufficient quantities and of a quality suitable for the consumer population.

INDEX TERMS: Mastitis, dairy goats, Minas Gerais, farms, risk factors, bacteria, *Staphylococcus aureus,* intramammary infection, small ruminants, antimicrobials, bacterioses.

RESUMO.- [Mastite em cabras de leite no estado de Minas Gerais: perfil de propriedades, fatores de risco e caracterização de bactérias.] A Zona da Mata de Minas Gerais possui uma cadeia especializada de produção de leite de cabra. O leite de cabra é superior em qualidade em comparação com o leite de outras espécies domésticas, e a demanda por leite e produtos lácteos do público tem aumentado. Os dados sobre o sistema de criação de cabras leiteiras em Minas Gerais são escassos e relativamente antigos, e essa falta de informação limita a implementação de medidas profiláticas, especialmente para a mastite, que representa o maior problema sanitário nos rebanhos leiteiros. Isso também causa problemas socioeconômicos e problemas de mercado para a criação de cabras leiteiras. O objetivo deste trabalho foi caracterizar a mastite e as bactérias associadas em cabras leiteiras na Zona da Mata de Minas Gerais. Um total de 539 cabras em lactação foi examinado e 268 amostras individuais (uma por teto) foram coletadas de animais positivos no teste da caneca de fundo escuro e/ou Califórnia Mastitis test (CMT). As culturas microbiológicas foram realizadas em meio Agar sangue e as bactérias foram submetidas a testes fenotípicos, genotípicos e testes de susceptibilidade antimicrobiana. A prevalência de mastite subclínica foi de 28,0% e a prevalência clínica foi de 2,8%. A multiplicação bacteriana foi obtida em 62,0% das amostras. Cento e oitenta e sete bactérias foram identificadas. As espécies mais identificadas foram: *Staphylococcus aureus* (60,4%), seguida de *Staphylococcus epidermidis* (9,1%,), *Escherichia coli* (6.9%), *Staphylococcus saprophyticus* (5.9%) e *Staphylococcus caprae* (4,3%) em ordem decrescente. As bactérias do gênero *Staphylococcus* apresentaram um perfil de resistência aos antimicrobianos pertencentes à classe de beta-lactâmicos - penicilina, ampicilina e oxacilina - além da tetraciclina, em contraste com os outros antimicrobianos testados. Doze por cento dos isolados apresentaram resistência múltipla a antibióticos (MDR) e foram encontrados em cinco microrregiões. Entre as bactérias com maior prevalência de MDR, 38,5% foram *E. coli* e 10,6% *S. aureus*. Os produtores da Zona da Mata de Minas Gerais são tecnificados, trabalham com raças leiteiras especializadas praticam e possuem bom manejo. No entanto, algumas medidas relacionadas à profilaxia e ao controle das doenças, como a vacinação, têm baixa adesão ou não são realizadas por falta de assistência veterinária. Este é o primeiro estudo com foco nesta região, que possui grande relevância na produção de leite de cabra no Brasil, fornecendo informações importantes que podem auxiliar na implementação de medidas de profilaxia e controle das doenças, e na manutenção de um fornecimento constante de produtos em quantidade e qualidade suficientemente adequada para a população consumidora.

TERMOS DE INDEXAÇÃO: Mastite, cabras de leite, Minas Gerais, propriedades, fatores de risco, bactérias *Staphylococcus aureus,* infecção intramamária, pequenos ruminantes, antimicrobianos, bacterioses.

INTRODUCTION

The world production of goat's milk is around 18.4 million tons per year contributing 2% of total milk production (FAO 2017). Brazil has a low production of about 35 million liters. The Northeastern region has the largest goat population in the country (92.6%), however, it produces less than 45% of the national total and consumption occurs predominantly in the producing areas (IBGE 2006). Production has been stimulated through the establishment of government programs for the purchase and pasteurization of goat's milk, with commercialization at or near the producer center (Gomes 2016).

The Southeast region produces most of the nation's goat milk, with more than 50% of the total, and its supply is concentrated in the states of Minas Gerais, São Paulo and Rio de Janeiro (Nogueira Filho et al. 2010). Minas Gerais produced three million liters in the year 2006, from 5835 milked goats (IBGE 2006). Current data shows that about 50,000 liters per day are produced in each state. However, since the supply is localized and quantities per producer are small, most producers market the food directly to the consumer, mainly in the form of milk and gourmet cheeses (Guimarães & Cordeiro 2017).

The Zona da Mata of Minas Gerais, composed of seven microregions, hosts approximately 20% of the national herd, and has animals specialized for milk production, obtained by the import of animals and semen, resulting in the most specialized goat milk production chain in Brazil (Fonseca & Bruschi 2009).

Goat milk is superior in quality compared with milk of other domestic species, and consumption by the public has increased considerably due to its nutritional properties. Goat's milk has a higher content of proteins, minerals and vitamin A, and presents smaller fat globules, facilitating the digestion process. It also has a lower lactose content and can be consumed by people who are lactose intolerant or have allergies to milk of other species (Nogueira Filho et al. 2010).

However, some factors limit this activity, especially mastitis which represents the major sanitary problem for dairy herds. In a study carried out by Gouveia et al. (2015), the prevalence of mastitis in dairy goats in the state of Minas Gerais was 41.7%.

Data on dairy goat breeding in Minas Gerais are scarce and relatively old; this restricted information has limited the implementation of prophylactic, socioeconomic and market measures in the industry (Gouveia et al. 2015).

Studies involving the etiology of caprine mastitis and resistance profiles of bacteria isolated from animals suffering the disease are scarce. Therefore, this study is necessary to better understand the factors inherent to the microorganisms that may be involved in the development of the disease.

The objective of this work was to characterize mastitis and bacteria associated with it in milking goats in the Zona da Mata of Minas Gerais.

MATERIALS AND METHODS

Ethics statement. The project was approved by the Committee of Ethics and Use of Animals of the Federal University of Viçosa, CEUA/UFV, with the number 42/2014.

Selection of farms and animals. The collections were carried out from April to August 2014. Ten properties were sampled in six micro-regions, since there were no dairy goats in one micro-region (Ponte Nova) at the time of collection. Twenty four of the properties with dairy goats in each microregion were selected and sampled. The Open Epi® program (http://www.openepi.com) was used, considering an estimated prevalence of 5%, an accuracy of 4% and a 95% confidence interval (Fig.1).

Diagnosis. For detection of clinical mastitis were performed physical examination and the dark background mug. For detection

subclinical mastitis were used the California Mastitis Test (CMT) score on a scale of 2, or 3 and was collected samples for confirmation by microbiological examination, one for each half udder (Peixoto et al. 2010).

Isolation and phenotypic identification of bacteria. All of the milk samples were obtained from cases of clinical and subclinical mastitis. After discarding a few first streams, about 15ml of milk individual sample were collected from each teat as recommended by the National Mastitis Council (NMC 2001). For the isolation and identification of the bacteria, 100μL samples of pre-homogenized milk were inoculated into Petri dishes containing 5% sheep blood agar (Oxoid) using the spread place method. These plates were then incubated at 37°C, and readings were performed after

24, 48 and 72 hours. The bacteria were later identified by their morphological, dyeing and biochemical characteristics (Quinn et al. 2011). The sample was considered positive for mastitis when three or more identical colonies were identified from an individual milk sample from a farm building (Buelow et al. 1996).

Genotypic identification. For DNA extraction, the Promega® kit was used, following the manufacturer's protocol. PCR was performed using the primers and amplification conditions described in Table 1. The amplified fragments were sent to Macrogen Corporation (Seoul, South Korea) for sequencing.

Resistance profile. Thirteen antimicrobials normally prescribed in the treatment of mastitis: ampicillin (10μg), neomycin (30μg), oxacillin (10μg), penicillin G (10 IU), enrofloxacin (10μg), ciprofloxacin
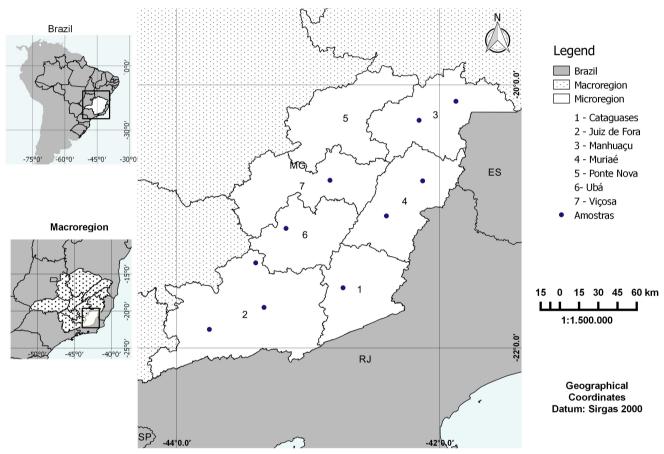

Fig.1. Location of the mesoregion Zona da Mata of Minas Gerais, Brazil, with microregions. The black dots show the locations of sample collection. The microregion of Ponte Nova (5) is not marked with black dots because there were no lactating goats at the time of collection.

Table 1. Primers used to identify bacteria isolated from goat milk with clinical and subclinical mastitis

Gene	Sequence (5′→3′)	Fragement size (bp)	Bacteria	Reference
se-1	ATCAAAAAGTTGGCGAACCTTTTCA	124	*Staphylococcus epidermidis*	(Martineau et al. 1996)
se-2	CAAAAGAGCGTGGAGAAAAGTATCA			
fema-1	AAAAAGCACATAACAAGCG	132	*Staphylococcus aureus*	(Martineau et al. 1998)
fema-2	GATAAAGAAGAAACCAGCAG			
616-1	AGAGTTTGATYMTGGCTCA	1530	rDNA 16S	(Sterr et al. 2009)
630-1	AAGGAGGTGATCCARCC			
uspa-1	CCGATACGCTGCAATCAGT	884	*Escherichia coli*	(Chen & Griffiths 1998)
uspa-2	ACGCAGACCGTAGGCCAGAT'			

(10µg), gentamicin (30µg), ceftiofur (30µg), sulfadiazine+Trimethoprim (20µg+1.25µg), cephalexin (30µg), cefepime (30µg), and cefaperazone (75µg) were used. Disc diffusion (Kirby-.Bauer) method was used following the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2008) was followed. Multidrug resistant isolates (MDR) were those that showed resistance to three or more classes of antimicrobials (Magiorakos et al. 2012).

Mastitis prevalence. The frequencies of positive and negative samples were tabulated in order to verify the association of the results using the chi-square test. To calculate the prevalence, the number of existing cases of mastitis (clinical and subclinical) divided by the total number of animals examined (total lactating females) was used.

Profile of properties and risk factors. A questionnaire was used to characterize the farm properties and to determine the risk factors. The questionnaire was applied with consent of the owner and the interviewee was always the person responsible for the management of the animals.

Statistical analyses. For the microbiological data, descriptive statistics were used to show the distributions of relative and absolute frequencies. For the analysis of the data related to risk factors for bacterial mastitis, association between the variables was tested using univariate and multivariate analysis.

In the univariate analysis, each independent variable was tested with the dependent variable (infection of the mammary gland). A 95% confidence interval was adopted using the EpiInfo program version 3.5.4.

For multivariate analysis, logistical regression was used. The response variables were the number of clinical and subclinical mastitis cases and the number of lactating females (trials). The explanatory variables were grouped according to their characteristics (related to hygiene, facilities, production and reproductive management, and the number of animals). Redundant explanatory variables were eliminated from the analysis to avoid severe multicollinearity. Only explanatory variables with significance at 10% probability were maintained in the final model.

RESULTS

Animals. A total of 539 lactating goats totaling 1078 half udder were examined, and 268 individual milk samples (one per farm building) were collected.

Identification of bacteria. One hundred and eighty-seven isolates were identified. The most prevalent genus was *Staphylococcus*, and a predominance of Gram-positive bacteria over Gram negative bacteria was also observed. *Staphylococcus aureus* was the most commonly identified species (60%, 113/187), followed by the coagulase negative *Staphylococcus* species (CNS) identified in 32% of cases: *S. epidermidis* (9%, 17/187), *S. saprophyticus* (6%, 11/187), *Staphylococcus* spp. (4.3%, 8/187), *S. caprae* (4.3%, 8/187), and *S. lugdunensis* (1.6%, 3/187). Other species of Gram positive bacteria were also identified: *Bacillus* spp. (0.5%, 1/187), *Bacillus vietnamensis* (0.5%, 1/187) and *Enterococcus faecalis* (0.5%, 1/187).

Among Gram-negative bacteria four members of the family Enterobacteriaceae were identified: *Escherichia coli* (6.9%, 13/187), *Klebsiella* spp. (2.1%, 4/187), *Klebsiella oxytoca* (0.5%, 1/187), *Enterobacter aerogenes* (2.1%, 4/187) and *Leclercia adecarboxylata* (0.5%, 1/187). Two other species, *Mannheimia glucosida* (0.5%, 1/187) from the family Pasteurellaceae, and *Acinetobacter* spp. (0.5%, 1/187) of the Moraxellaceae family were also found.

In clinical mastitis, two species were identified: *S. aureus* in 86.7% of cases (13/15) and *E. coli* in 13.3% (2/15). Figure 2 shows the bacteria isolated and identified in the studied microregions. In all microregions the genus *Staphylococcus* predominated. *S. aureus* was the most isolated species in each microregion, with the exception of the Cataguases microregion where 70% of the isolates were coagulase negative staphylococci (CNS).

In the microregions of Ubá and Cataguases, only bacteria of the genus *Staphylococcus* were found, while in the microregions of Viçosa, Juiz de Fora, Manhuaçu and Muriaé, other species were found including members of the family Enterobacteriaceae (Fig.2).

Antimicrobial resistance. Bacteria of the genus *Staphylococcus* presented a profile of resistance to the beta-lactam class of antimicrobials: penicillin, ampicillin and oxacillin, in addition to tetracycline, compared with the other antimicrobials tested (Table 2).

S. aureus presented resistance to several classes of antimicrobials, with resistance to penicillin, ampicillin and oxacillin occurring in all microregions studied. Resistance to penicillin ranged from 12.5% to 66.6%, while ampicillin and oxacillin resistance ranged from 12.5% to 100% among the microregions (Table 2).

Gram-negative bacteria had fewer resistant isolates. Among the isolates of *E. coli*, the following percentages of resistant isolates were found: beta-lactam (23% ampicillin), cephalosporins (30.8% cephalexin and 7.7% cefepime) tetracycline (tetracyclin 23%) and sulfonamides (7.7% sulfadiazine+trimethoprim). Among *Klebsiella* spp. and *Acinetobacter* spp. isolates, most were sensitive to most antimicrobials, with resistance only shown to ampicillin.

Bacillus spp., *Bacillus vietnamensis*, *Mannheimia glucoside* and *Enterococcus faecalis* isolates showed 100% sensitivity to all tested antimicrobials.

Twelve (12%, 20/166) of mutli-resistant drug (MDR) isolates were found in five microregions. Among the bacteria with the highest prevalence of MDR 10.6% were *S. aureus* and 38.5% were *E. coli.*

In the Cataguases microregion, a *S. saprophyticus* MDR isolate with resistance to beta-lactams (penicillin, ampicillin and oxacillin), tetracyclines (tetracycline), and cephalosporins (cefoperazone and cefepime) was found.

In the Manhuaçu microregion *S.aureus* and *E. coli* MDR isolates were found. There were two *S. aureus* MDR isolates resistant to four classes: aminoglycosides (neomycin), quinolones (ciprofloxacin), beta-lactams (penicillin and oxacillin), and cephalosporins (cephalexin).

An *E. coli* MDR isolate showed resistance to four classes: sulfonamides (sulfadiazine+trimethoprim), quinolones (enrofloxacin), tetracyclines (tetracycline), and cephalosporins (cefatiofur, cephalexin and cefepime).

In the Muriaé microregion MDR isolates of the species *S. aureus*, *S. epidermidis* and *E. coli* were found. Five *S. aureus* MDR isolates were found that were resistant to three classes: aminoglycosides (gentamicin), beta-lactams (penicillin and oxacillin), and cephalosporins (cephalexin). A MDR isolate of *S. epidermidis* was resistant to three classes: tetracyclines (tetracycline), beta-lactams (penicillin, ampicillin and oxacillin), and cephalosporins (cefepime). An *E. coli* MDR isolate was resistant to five classes: aminoglycosides (gentamicin and
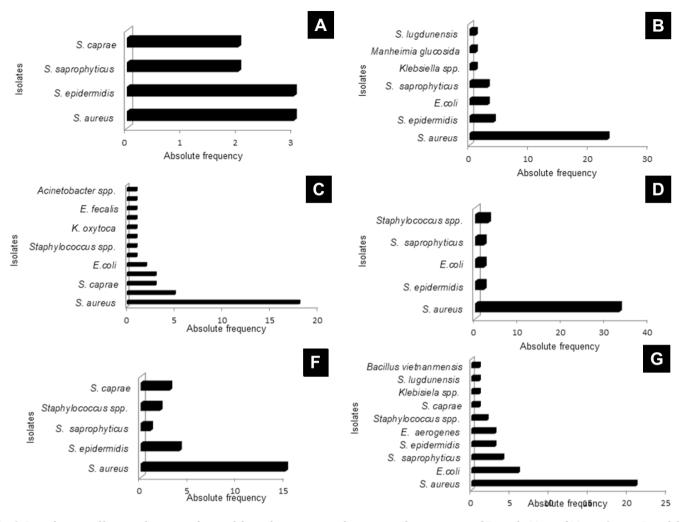
Fig.2. Distribution of bacterial species obtained from dairy goats with mastitis of mesoregion of Zona da Mata of Minas Gerais, Brazil, by microregion. Microregions: Cataguases (**A**), Juiz de Fora (**B**), Manhuaçu (**C**), Muriaé (**D**), Ubá (**F**) and Viçosa (**G**). (**E**) represents the microregion of Ponte Nova which was not collected samples.

neomycin), tetracyclines (tetracyclines), cephalosporins (cefatiofur), quinolones (enrofloxacin) and sulphonamides (sulfadiazine+trimethoprim).

In the micro-region of Juiz de Fora, three MDR isolates were found. An *E. coli* isolate was resistant to four classes: beta-lactams (ampicillin), cephalosporins (cefepime, cefatiofur and cephalexin), quinolones (enrofloxacin) and sulphonamides (sulfadiazine+trimethoprim). An *S. epidermidis* isolate was resistant to four classes: aminoglycosides (gentamicin and neomycin), tetracyclines (tetracyclines), beta-lactams (ampicillin, penicillin and oxacillin), and cephalosporins (cefatiofur). An isolate of *S. saprophyticus* was resistant to four classes: aminoglycosides (gentamicin), tetracyclines (tetracyclines), beta-lactams (ampicillin, penicillin and oxacillin), and cephalosporins (cefepime).

In the micro-region of Viçosa, five MDR *S. aureus* isolates were found that were resistant to four classes: aminoglycosides (gentamicin and neomycin), tetracyclines (tetracyclines), beta-lactams (penicillin and oxacillin), quinolones (ciprofloxacin)

and cephalosporins (cephalexin). No MDR isolates were found in the Ubá micro-region.

Prevalence of mastitis. One hundred and sixty-six animals were positive for mastitis, representing 62% (166/268) of the samples obtained (i.e. with bacterial multiplication, and three or more identical colonies; (Buelow et al. 1996). Of these, 91% (151/166) were cases of subclinical mastitis and 9% (15/166) were clinical mastitis. In 38% (102/268) of the samples there was no bacterial multiplication, and they were considered negative for mastitis.

The prevalence of subclinical mastitis in properties housing dairy goats in the Zona da Mata of Minas Gerais was 28% (151/539), and the prevalence of clinical mastitis was 2.8% (15/539). The prevalence of subclinical mastitis per microregion varied between 17% and 59% (Table 3). Clinical mastitis was detected in only four microregions, where the prevalence ranged from 1.3% to 11.1% (Table 3).

In properties where manual milking was performed the prevalence of mastitis was 53.2%, while in those that used mechanical milking the prevalence was 25.5%. The prevalence

Table 2. Distribution of bacterial species obtained from dairy goats with mastitis of mesoregion of Zona da Mata, Minas Gerais, Brazil, by microregion

Antimicrobial class	Antimicrobial	*Staphylococcus aureus* No. of isolates (%)			*Staphylococcus epidermidis* No. of isolates (%)			*Staphylococcus saprophyticus* No. of isolates (%)			*Staphylococcus* spp. No. of isolates (%)			*Staphylococcus caprae* No. of isolates (%)			*Staphylococcus lugdunensis* No. of isolates (%)		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Beta-lactams	PEN	43 (38.0)	–	70 (62.0)	9 (53.0)	–	8 (47.0)	4 (40.0)	–	6 (60.0)	1 (12.5)	–	7 (87.5)	3 (37.5)	–	5 (62.5)	2 (66.6)	1 (33.3)	–
	AMP	30 (26.5)	–	83 (73.5)	3 (17.6)	–	14 (82.4)	–	–	10 (100)	–	–	8 (100)	1 (12.5)	–	7 (87.5)	–	–	3 (100)
	OXA	29 (25.6)	–	84 (74.4)	3 (17.6)	–	14 (82.4)	–	–	10 (100)	1 (12.5)	–	7 (87.5)	–	–	8 (100)	–	–	3 (100)
Aminoglycosides	GEN	14 (12.3)	–	99 (87.7)	2 (11.8)	–	15 (88.2)	1 (10.0)	–	9 (90.0)	–	–	8 (100)	–	–	8 (100)	–	–	3 (100)
	NEO	10 (8.8)	2 (1.8)	101 (89.4)	3 (17.6)	–	14 (82.4)	2 (20.0)	–	8 (80.0)	–	–	8 (100)	–	–	8 (100)	–	–	3 (100)
Quinolones	ENRO	–	–	113 (100.0)	–	–	17 (100.0)	–	–	10 (100.0)	–	–	8 (100)	–	–	8 (100)	–	–	3 (100)
	CIP	10 (8.8)	–	103 (91.2)	–	–	17 (100.0)	–	–	10 (100.0)	–	–	8 (100)	–	–	8 (100)	–	–	3 (100)
Cephalosporin 1st generation	CFX	11 (9.7)	–	102 (90.2)	7 (41.2)	–	10 (58.8)	–	–	10 (100)	–	–	8 (100.0)	–	–	8 (100)	–	–	3 (100)
Cephalosporin 3rd generation	CFT	3 (2.7)	5 (4.4)	105 (92.9)	2 (11.8)	–	15 (88.2)	–	–	10 (100)	–	–	8 (100)	–	–	8 (100)	–	–	3 (100)
	CFP	4 (3.5)	5 (4.4)	104 (92.1)	2 (11.8)	–	15 (88.2)	–	–	10 (100)	–	–	8 (100)	–	–	8 (100)	–	–	3 (100)
Cephalosporin 4th generation	CFM	2 (1.8)	–	111 (98.2)	–	–	17 (100)	–	–	10 (100)	1 (12.5)	–	7 (87.5)	–	–	8 (100)	–	–	3 (100)
Sulfonamides	SUT	11 (9.7)	1 (0.9)	101 (89.4)	–	–	17 (100.0)	1 (10.0)	–	9 (90.0)	–	–	8 (100)	–	–	8 (100)	–	–	3 (100)
Tetracycline	TET	19 (16.8)	–	94 (83.2)	6 (35.3)	–	11 (64.7)	3 (30.0)	–	7 (70.0)	2 (25.0)	–	6 (75.0)	2 (25.0)	–	6 (75)	–	–	–

Microregions: Cataguases (1), Juiz de Fora (2), Manhuaçu (3), Muriaé (4), Ubá (6) and Viçosa (7). PEN = Penicilin, AMP = Ampicilin, OXA = Oxacilin, GEN = Gentamicin, NEO = Neomicin, ENRO = Enrofloxacin, CIP = Ciprofloxacin, CFX = Cephalexin, CFT = Ceftiofur, CPF = Cefoperazone, CFM = Cefepime, SUT = Sulfadiazine + Trimethoprim, TET = Tetracycline, R = resistence, S = sensible, I = intermediar.

of mastitis was lower when milking was performed on a platform (27.5%), whereas in those properties where the animals were milked in the corral itself, a higher frequency of positive animals (59%) was found.

Risk factors. The frequency of cleaning of drinking fountains, the type of flooring, an interval between deliveries (<12 months), the percentage of lactating females, and manual milking all had significant effects on the prevalence of mastitis (Table 4).

Cleaning of drinking water fountains less frequently (<2 times a week) increased the risk of subclinical mastitis by 2.75 times and the risk of clinical mastitis by 8.6 times. Properties where manual milking was performed were 4.93 times more likely to have cases of subclinical mastitis. The type of facility used for lactating females was a risk factor for clinical and subclinical mastitis in this study.

The use of slatted flooring increased the risk of subclinical mastitis 1.98 times, whereas the use of bedrock increased the risk of clinical mastitis 2.83 times. Manual milking was shown to be a risk factor for subclinical mastitis in multivariate analysis, while an effect of the type of milking on clinical mastitis was not observed.

Property profile. The farm properties had mechanical (60%) and manual (40%) milking systems, housing 22 to 108 lactating goats. The animals were of the Saanen and Alpine breeds. Ten people were interviewed, 70% (7/10) were owners, 20% were tenants (2/10) and 10% (1/10) were employees. Their average age was 42 years and all were males. Sixty percent of the interviewees had worked with goats for more than 13 years and 40% for less than 10 years.

All respondents (10/10) had at least completes elementary education, while 40% (4/10) had started and 30% (3/10) had completed higher education. Goat farming was considered

the main activity for 50% (5/10) of the interviewees. Other concomitant activities observed were the production of cachaça, coffee and cow's milk. In this study, the farms were predominantly small properties, 80% (8/10) had an average of 10 hectares. All of the sampled properties were focused on the production of goat milk and sold the milk to a dairy or directly to the market/consumer (milk or milk products). The average price was R$ 1.67 per liter (in 2014). In all of the properties the animals were raised in an intensive system. Dairy goats were considered profitable by 70% (7/10) of the interviewees. There was at least one employee on 60% of the properties.

The main breed found in the herds studied was Saanen (90%), and in three farms there were also Alpine goats. One farm (10%) owned exclusively brown animals. There were, on average, 60 lactating goats per farm. The mean lactation period was 282.5 days, with 70% of the properties keeping the animals in production for at least 300 days. The overall average milk yield was 140.25 liters and the average daily production was 2.55 L/animal/day. Saanen goats averaged 2.73 L/animal and Alpine goats averaged 2.3 L/animal. All of the properties subjected the animals to two milkings per day.

The lactating animals were housed in suspended slat facilities in 70% of the properties while 30% used beds, where sawdust was used in 20% and sugarcane bagasse in 10% of properties. In 90% (9/10) of the properties, the animals were individually identified, with 44% wearing collars, 22% being tattoo, 11% wearing earrings, and in 22% of the properties a combination of tattoos and collars were used.

In all of the properties, newborn animals were fed with goat's milk (40%), cow's milk (40%), milk powder of either species (10%) or a substitute (10%). The navel was healed in 90% of the properties, using 10% iodine solution. Only in

Table 3. Prevalence of clinical and subclinical mastitis in dairy goats, by microregion that compose the mesoregion of Zona da Mata, Minas Gerais, Brazil

Microregion	No. of properties	No. of animals examined	% Subclinical mastitis	% Clinical mastitis
Viçosa	1	108	27.7% (30/108)	11.1% (12/15)
Ubá	1	34	52.9% (18/34)	1.3% (1/15)
Juiz de Fora	3	219	17.0% (37/219)	–
Manhuaçu	2	77	29.8% (23/77)	1.30% (1/15)
Muriaé	2	78	38.5% (30/78)	1.30% (1/15)
Cataguases	1	22	59.0% (13/22)	–
TOTAL	10	539	28.0% (151/166)	2.8% (15/166)

Table 4. Multivariate analysis with the distribution of variables related to management and associated with risk factors for mastitis in dairy goats from Zona da Mata, Minas Gerais, Brazil

Explanatory variables	Subclinical mastitis			Clinical mastitis		
	P^a	RC^b	IC^c	P^a	RC^b	IC^c
Frequency of cleaning of drinking fountains, more vs less than 2 times per week	<.0001	2.755	1.675-4.532	0.0395	8.612	1.109-66.853
Type of milking, manual vs. mechanical	<.0001	4.933	2.762-8.813	NS	-	-
Installation, floor vs bed	0.0010	1.980	1.318-2.976	0.0645	0.352	0.117-1.065
Interval between births, <12 months vs >12 months	NS	-	-	.0046	6.579	1.789-24.390
Age at first birth in months	<.0001	1.499	1.258-1.789	NS	-	-
Percentage of lactating females	<.0001	6.289	2.778-14.286	0.0018	500.00	9.52->1000

[a] Value of P, [b] odds ratio, [c] confidence interval with 95%; NS = not significant (*P*>0.05).

20% of the properties did the water used for the consumption of the animals come from the public supply system. In the rest of the properties, alternative sources of water were used: 30% from artesian wells, 20% from mine water, 20% from semi-artesian wells and 10% from springs. Cleaning of drinking fountains was carried out on all properties, with varying frequencies. Only 10% of drinking water of animals were cleaned daily, 30% were cleaned once a week and 30% were cleaned twice a week, and 30% were not cleaned or the interviewees did not know how to frequency of clean.

Manual milking was performed in 40% of the properties, of which 20% did not perform hygiene using pre- and post-dipping solutions, and one of them did not use a milking parlor, the animals being milked inside the stalls. All properties that performed mechanical milking (60%) maintained a hygiene routine with pre- and post-dipping solutions.

Ninety percent of the properties did not have veterinary assistance. The animals were vaccinated in only 40% of the properties: 10% used only the vaccine against clostridiosis, 20% inoculated against clostridiosis and rabies, and 10% used three vaccines, against clostridiosis, rabies and leptospirosis. No properties used the vaccine against caseous lymphadenitis, and the disease was reported in 70% of the properties. An isolation area for diseased animals was absent in 30% of the establishments.

Cleaning of the facilities with slatted floors was performed using a broom to remove the feces. Exchanging the bedding on beaten floors was carried out in conjunction with the disinfection of the premises. These hygiene procedures occurred at varying frequencies among the properties: in 40% (4/10) they were carried out every two months, in 20% (2/10) every six months, and in 10% (1/10) every 12 months. In 10%, the procedures were performed once a week or twice a month, and one respondent was not able to answer. The most commonly used disinfection method was the use of a lime-associated fire broom (60%). Other methods used were: lime only (10%), fire broom only (20%) and lime associated with quaternary ammonia solution (10%).

DISCUSSION

Mastitis is an important disease in livestock farming systems, especially in dairy animals, due to the damages it causes by reducing the production and quality of the milk produced, as well as incurring the costs of treatment, labor and milk disposal (Contreras et al. 2007). The high prevalence of *S. aureus* in goat's milk may pose a risk to the health of consumers. It is known that *S. aureus* produces large amounts of exotoxins known to cause staphylococcal food poisoning, and it may be involved in other types of infections in humans and animals. Since many traditional products originating from goats are not subjected to pasteurization, this may be a potential source of food poisoning (Merz et al. 2016).

Samples of milk from goats positive in the CMT test but without bacterial isolation, must be carefully analyzed during the diagnosis of subclinical mastitis in goats to minimize false-positive reactions (Bianchini et al. 2010). The presence of other etiological agents that cannot be isolated in culture medium normally used for routine microbiological examinations, such as *Mycoplasma* (Kinde et al. 1994) and non-bacterial infectious agents like caprine arthritis encephalitis virus

(CAE) (Gregory et al. 2009) and fungi (Jensen et al. 1996) must be considered.

The state of Minas Gerais pioneered dairy goat farming in Brazil by importing specialized breeds from different European countries, the United States and Canada, to improve the genetic potential and milk production in local herds (Fonseca & Bruschi 2009). The Zona da Mata of Minas Gerais is a mesoregion of great importance in the production of goat's milk in the Southeast, due to its high productivity and use of technology comparing to other states of Brazil.

In this study, the most commonly identified bacteria belonged to the genus *Staphylococcus*, with *Staphylococcus aureus* being the most frequently isolated species. This was similar to studies carried out in other countries, where animals are bred in similar conditions in Pakistan (Najeeb et al. 2013) and in Italy (Ceniti et al. 2017).

In this study, we found 27.7% (46/166) of CNS isolates, and differing from studies performed in other regions of Brazil, where they found CNS in 83.3% (Neves et al. 2010) and 79.7% of isolates in the Northeast region (Cavalcante et al. 2013), and 62.2% (Almeida et al. 2013) and 90.5% (Gomes et al. 2014) in the Southeast region. In Brazil, only phenotypic methods have been used in the identification of bacterial species from caprine mastitis samples in the studies published so far. Genotypic methods have a higher degree of discrimination (Ruegg 2009), which may explain the differences found in this study, since we used PCR and sequencing in addition to phenotypic tests.

Mastitis was considered the main sanitary and economic problem by producers in 30% of the dairy goat properties in the Zona da Mata of Minas Gerais, and we detected a similar prevalence of mastitis in the microregions studied; however, in some microregions the prevalence of mastitis was above that threshold. This may be because most mastitis manifests in the subclinical form and the producers do not detect it. In the properties where manual milking was carried out, the prevalence of mastitis was higher, so we can infer that type of milking may be a problem. This information is important for planning measures to control mastitis in dairy herds, such as improvement in the hand hygiene of milkers.

In a study conducted by Salaberry et al. (2016) in relation to the resistance profile, *Staphylococcus* isolates from goats with mastitis in the Southeast region of Brazil presented greater resistance to penicillin (81.8%), oxacillin (60.0%) and ampicillin (55.5%) in agreement with the data obtained in this study. A study conducted by Teshome et al. (2016) in dairy goats in Ethiopia similarly found 78.9% of *S. aureus* samples were penicillin resistant.

França et al. (2012) also studied *Staphylococcus* spp. finding that isolates were more resistant to amoxicillin (50.0%), streptomycin (43.4%), tetracycline (40.5%), oxacillin (16.7%) in sheep and goat mastitis samples from the Northeast region of Brazil. There has greater resistance to gentamicin (7.62%) in goat isolates compared to sheep isolates.

Although the results in relation to the resistance profile found in this study are in general agreement with previous research, comparison between different studies is difficult due to different methods of susceptibility testing and the interpretative criteria used to categorize isolates as susceptible or resistant. Antimicrobial tests are useful for detecting the most efficient drugs, although several factors may influence

the general susceptibility pattern of mastitis pathogens (Oliver et al. 2011).

In this study was found the low percentage of MDR strains (12%) in relation to other studies was found. Even so, this data is relevant because it can interfere in the treatment of mastitis, besides the possibility of transfer of resistance genes to other bacteria. In a study carried out with several bacteria from caprine mastitis samples in Pakistan, 44% were MDR, considering two classes of antibiotics (Najeeb et al. 2013). In a study conducted in China, 39.7% of MDR *S. aureus* isolates obtained from goat's milk was resistant to four antimicrobials. In Ethiopia high MDR (69.2%) rates were found in *S. aureus* isolates from goat milk, which were resistant to two classes of antimicrobials (Teshome et al. 2016). The likely explanation for the high degree of multidrug resistance found in these studies is the repeated use of antibiotics in animals and humans in those regions and countries.

The prevalence of subclinical mastitis found in this study was 28%, in agreement with other study conducted in Brazil, the Southeast region by Gomes et al. (2014) and the northeastern region by Bianchini et al. (2010) and Peixoto et al. (2012). In Italy, Dore et al. (2016) also found the similar prevalence. All of these studies were performed with goats of the same breeds and with a similar breeding system.

However Neves et al. (2010) found the prevalence of subclinical mastitis (11.49%) lower than in this study, possibly due to the different production system (extensive) where the possibility of contamination is minor

Clinical mastitis occurred in only four microregions, and in three the prevalence was 1.3%, being within the 5% limit considered acceptable (Contreras et al. 2007). This was similar to recent studies carried out in the northeastern region of Brazil, where the prevalence of reported clinical mastitis was 3.4% in goats bred for meat and milk (Cavalcante et al. 2013) and 0.15% in goats of the same breeds and breeding system as the present study (Bianchini et al. 2010).

In only one microregion was the prevalence of clinical mastitis (11.1%) above the acceptable limit (5%). However, although this property used mechanical milking, it have turnover of employees could explain the difficulty of standardization of mastitis control and prophylaxis measures.

The risk factors found for mastitis in this study differed from those found in the few similar studies done in Brazil (Neves et al. 2010, Peixoto et al. 2012, Gomes et al. 2014). These studies relate to risk factors for caprine mastitis in the northeast region of the country, where the breeds, management and climatic conditions are different. This makes it difficult to compare the frequencies of bacterial isolation and risk factors with other Brazilian regions. In the southeast region, differently from the northeast, the animals tend to be raised in a more intensive regime with greater use of technology.

In this study, although the producers are well educated, and work with animals of breeds specialized for milk production using good management, some measures, mainly related to prophylaxis and control of the diseases have low adherence or are not yet established. This is likely due to the lack of veterinary assistance, as there are few veterinarians specializing in goats in the region.

The lack of veterinary assistance is reflected in the prophylactic management of diseases, since only 40% of the properties vaccinated their animals. Although the producers have a good level of education the majority do not have training in the area, and this makes it difficult for them to understand the importance of measures such as vaccination. We found similar data to those found by Gouveia et al. (2015) in a study conducted in the State of Minas Gerais, where 50% of dairy farmers used some type of vaccine, 48% used a clostridial vaccine, 1.2% vaccinated against caseous lymphadenitis, and 23.8% against rabies. However, no properties in the present study used the foot-and-mouth disease vaccine in the Zona da Mata of Minas Gerais, while Gouveia and collaborators (2015) found that 20.2% of properties used this vaccine, ignoring the prohibition of goat vaccination by the Ministério da Agricultura Pecuária e Abastecimento (MAPA). In this study, no property used the vaccine against caseous lymphadenitis, a disease reported in 70% of the properties. This is probably due to a lack of information, or even inadequate use of the vaccine in animals that already have the disease in their internal lymph nodes or are in the incubation period, and thus present the symptoms of the disease after vaccination.

Most of the properties had at least one employee, which demonstrates the ability of the dairy goat to generate jobs and commercialize the milk, showing the economic importance of dairy goat for the mesoregion.

CONCLUSIONS

The prevalence of clinical mastitis was 2.8% and subclinical mastitis 28%.

Staphylococcus aureus was the most commonly isolated bacterium with a profile of resistance mainly to penicillin, ampicillin and oxacillin.

12% of the bacteria presented a multidrug resistant profile (MDR).

The farming of dairy goats is a significant activity in the Zona da Mata of Minas Gerais region.

This seems to be the first study focusing on mastitis at dairy goats in this region and provides information that will assist in measures of prophylaxis and control, that there are numerous challenges to maintaining a constant supply of goat milk and its derivatives in quantity and with good quality.

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