RESEARCH ARTICLE

SUBCLINICAL MASTITIS OF LACTATING SAHEL GOATS FROM URBAN SMALLHOLDER HERDS IN MAIDUGURI, NIGERIA: PREVALENCE AND INTRA-MAMMARY INFECTIONS

Daniel Thomas Yoksa[1], Dauda Luka Mohzo[1], Nanacha Afifi Igbokwe[2], Moses Ndiryizah James[1], Ikechukwu Onyebuchi Igbokwe*[1]

[1]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Maiduguri, Nigeria
[2]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Maiduguri, Nigeria

***Corresponding author:**
Prof. Dr. Ikechukwu Onyebuchi Igbokwe

Address: Strategic Animal Research Group, Faculty of Veterinary Medicine, P. O. Box 8000, UNIMAID PO, Maiduguri 60001, Nigeria
Phone: +234 8087706808
ORCID: 0000-0002-5039-4496

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ABSTRACT

Risk of chronic clinical mastitis could be increased by insidious subclinical mastitis (SCM) in urban smallholder goat herds. This study was a cross-sectional survey of apparently healthy lactating Sahel goats (February-March, 2023) for prevalence of SCM using California mastitis test (CMT) and bacterial culture of raw milk in Maiduguri, Nigeria. Positive CMT was graded as +1 (mild), +2 (moderate), or +3 (severe). Standard procedures for cultural isolation and identification of bacteria were followed. Prevalence of SCM was 38% (95% CI, 26.9-52.2) from 100 goats, with the parity-specific prevalence (18.7-75.0%) decreasing ($p < 0.05$) from 50.0% at 1st parity to 18.7% at 3rd parity and increasing ($p < 0.05$) to 75.0% at 6th parity. Parity was not significantly ($P > 0.05$) associated with the prevalence. Most of the cases (71.1%, 27/38) were mild. The CMT diagnosis was significantly ($p < 0.0001$) associated with bacterial isolation from the milk samples. Isolation rate was 92.1% (35/38) in SCM cases, but CMT negative milk samples had isolation rate of 4.8% (3/62). *Staphylococcus spp* and *Streptococcus spp* were isolated in pure culture in 26.3% and 10.5% of 100 samples, respectively. Both *Staphylococcus spp* and *Streptococcus spp* were isolated together in 63.2% of the samples. Out of 34 isolates of Staphylococcus spp, 24 (70.6%) were coagulase-positive. Staphylococci (89.5%, 34/38) and streptococci (73.7%, 28/38) isolation rates did not differ ($\chi2 = 3.118$; $p < 0.077$). Therefore, prevalent population of SCM cases among healthy lactating Sahel goats with intra-mammary infections should be controlled to prevent escalation to clinical mastitis.

Keywords: Subclinical mastitis, Sahel goat, *Streptococcus*, *Staphylococcus*, prevalence

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INTRODUCTION

Goats in Nigeria have a daily milk yield of 0.4-1.3 kg during a lactation period of 100-126 days (Akpa et al 2002), and improved goat milk production has been advocated (Egwu et al 1995), but the incidence of mastitis is among diseases limiting this goal (Abba et al 2013, 2014). Clinical mastitis affects about 8-17% of goats (Ameh et al 1993; Ameh and Tari, 1999; Danmallam and Pimenov, 2019) where clinical signs were identified with abnormalities in the produced milk. However, subclinical mastitis is usually not associated with visible abnormality in the udder or milk, but milk production could decrease, and raw milk secreted contains elevated number of somatic cells (Koop et al 2012). The diagnostic approach to identifying SCM involves somatic cell count in milk and bacterial culture of the milk, but increased somatic cell count may not be accompanied by bacterial culture. Therefore, somatic cell count and bacterial culture are done together for effective monitoring of SCM in goats (Koop et al, 2012). California mastitis test (CMT) is a screening test which detects the amount of deoxyribonucleic acid (DNA) in milk, correlating with the quantity of somatic cells in the milk, and could predict somatic cell count (Persson and Olofsson, 2011; Souza et al, 2012), which is helpful in detecting SCM with intra-mammary infection (McDougall et al, 2010).

In northern Nigeria, SCM of Sahelian goats was previously detected solely by bacterial culture of raw milk from apparently healthy udders, and the prevalence was 35-47% (Ameh et al, 1993; Ameh and Tari, 1999; Alawa et al, 2000); but CMT was used recently in the diagnosis of caprine SCM, and the prevalence was 23-60% in Kaduna, Bauchi and Plateau States (Udo et al, 2019; Danmallam and Pimenov, 2019). The reduction in milk production could lead to malnutrition of the kids causing poor growth and kid mortality due to starvation (Fthenakis and Jones, 1990). The mortality of goat kids was reported to be 41.4% and associated with diarrhea and starvation (Ameh et al., 2000). The infected milk could also be a source of infection to the kids resulting in gastroenteritis and diarrhea with subsequent mortality. The prevalence of SCM appears to be higher than clinical mastitis (Koop et al, 2012) and could persist in the herd without regular monitoring and adoption of preventive and control strategies. In Maiduguri (Borno State), Nigeria, prospective survey of SCM in lactating Sahel goats has not been reported recently and the surveillance seems to be inactive for the epidemiological monitoring and control of the disease. The objective of the observational study was to conduct cross-sectional survey of apparently healthy lactating Sahel goats for prevalence of SCM using CMT and bacterial culture of raw milk.

MATERIALS AND METHODS

Study design

The study was a non-randomized cross-sectional survey of apparently healthy lactating Sahel goats in smallholder farms in an urban area with the purpose of diagnosing SCM using CMT and bacterial culture of raw milk samples collected from clinically healthy mammary glands (udders) without gross evidence of inflammation. In order to exclude clinical mastitis during the survey, the selected goat was expected to have normal vital parameters and no abnormality after physical examination of the body and udder, and milk was considered normal when it had no gross abnormality in color, odor or consistency.

Selection of Sahel goats

The Sahel goats were selected by convenience sampling and snowball technique from 28 smallholder goat herds located within the township environment of Maiduguri (11.83°N, 13.15°E), Nigeria, during the months of February-March, 2023. The herds were housed within fenced premises and managed semi-intensively. The goats were fed cowpea husks, groundnut hay, sorghum and wheat offal, and offered water in their pens, but allowed to move around the vicinity of the homestead to browse on any available feedstuff (Figure 1). Physical examination was conducted to ascertain health status. The goats were aged by

dental examination (2.0-5.0 years), and were not weighed, but were estimated to be in good body condition. Rectal temperature was measured with digital thermometer and confirmed to be normal (37.5-39.0° C). The history of kidding was taken to find out parity and number of kids per kidding. Each doe had a recent kidding of 1-3 kids and was lactating.

Figure 1 An urban herd of Sahel goats in Maiduguri, Nigeria

Milk sample collection

Udder of the doe was disinfected using 70% alcohol and 2 - 3 squirts of milk were manually milked off and discarded, after which another 2-3 squirts were collected in a gloved hand to check the physical quality of the milk in terms of the color, consistency and odor. The raw milk was collected aseptically for CMT and bacterial culture in a sterile vial, and transported to the laboratory on a cold-chain facility.

California mastitis test (CMT)

Qualitative estimation of somatic cells infiltrated into the milk was determined using CMT (Leach et al, 2008). Briefly, the milk was collected into the paddle and mixed with equal amount of CMT liquid (BOVI-VET, KRUUSE, Denmark) by gentle movement of the hand. There was no gel formation in negative test, but positive (+1 [mild], +2 [moderate], +3 [severe]) tests were based on the intensity of gel formation. The paddle was washed and the procedure was repeated with another collected milk sample.

Cultural isolation and identification of bacteria from milk

Milk sample was inoculated by direct streaking onto 5% sheep blood agar, mannitol salt agar, and eosin methylene blue agar and incubated at 37 °C for 48 hr for the growth of bacterial colonies. The morphological characteristics of bacterial growth and hemolytic effect, the nature of Gram staining, and coagulase and catalase tests guided the identification of the bacterial isolate (NMC, 2004; Quinn, 2011).

Statistical analysis

The data were summarized descriptively as counted numbers and their proportions of the population tested. The overall prevalence was calculated as the proportion of the population testing CMT positive in either half or both halves of the udder (goat level), and the 95% confidence interval (CI) for the prevalence was calculated. The parity-specific prevalence was calculated as the positive proportion at each parity level; the comparison of parity prevalence (1-6 parity) was done with one-way chi-squared test. The two-way chi-squared test for trend was calculated by entering CMT negative and positive categories in one classification and parity in the second classification to determine relationship between parity and prevalence. Two-sided chi-squared with Yates correction was carried out to determine the association of CMT positive tests with bacterial isolation. All calculations and inferences were done with statistical software (www.medcalc.org/calc/ version 22.017; accessed February 2, 2024 or GraphPad InStat, GraphPad Software Inc., CA., USA, www.graphpad.com).

RESULTS

The overall prevalence of SCM, based on CMT, in Sahel goat population (n =100) was 38% (95% CI, 26.9-52.2%), with the parity-specific prevalence (18.7-75.0%) decreasing ($p < 0.05$) from 50.0% at 1st parity to 18.7% at 3rd parity and increasing ($p < 0.05$) to 75.0% at 6th parity (Table 1). The SCM was not observed at 7-9th parity. Parity was not significantly ($P > 0.05$) associated with the prevalence. Most of the cases of SCM (71.1%, 27/38) were mild, while moderate and severe SCM occurred in 18.4% (7/38) and 10.5% (4/38) of the prevalent cases, respectively. The CMT diagnosis of SCM was significantly ($p < 0.0001$) associated with the isolation of intra-mammary bacteria from the milk samples, with positive and negative predictive values of 92.1% and 95.2%, respectively (Table 2). The isolation rate was 92.1% (35/38) in CMT positive SCM cases; whereas CMT negative milk samples had isolation rate of 4.8% (3/62) of staphylococci. *Staphylococcus spp* and *Streptococcus spp* were isolated in pure culture in 26.3% and 10.5% of 100 samples, respectively. Both *Staphylococcus spp* and *Streptococcus spp* were isolated together in other 63.2% of the samples. Out of the 34 isolates of Staphylococcus spp, 24 (70.6%) were Coagulase-positive and identified phenotypically as S. aureus. The frequency of isolation of staphylococci (89.5%, 34/38) and streptococci (73.7%, 28/38) in SCM cases did not differ ($\chi 2 = 3.118$; $p < 0.077$). There was no *E. coli* isolation in any sample.

Table 1 Prevalence of subclinical mastitis in lactating goats based on California mastitis test

		Positive				
Parity	Negative, N	+1	+2	+3	N (%)	Total (N)
1	9	4	2	3	9 (50.0)*	18
2	25	17	2	0	19 (43.2)	44
3	13	2	1	0	3 (18.7)	16
4	4	0	0	1	1 (20.0)	5
5	5	1	2	0	3 (37.5)	8
6	1	3	0	0	3 (75.0)	4
7	2	0	0	0	0	2
8	2	0	0	0	0	2
9	1	0	0	0	0	1
	62	27	7	4	38 (38, 26.9-52.2)	100

N, number of goats; Prevalence (%) in parenthesis

*Significant ($p < 0.0001$) variation in parity-specific prevalence (one-way chi-squared)

Table 2 Association of subclinical mastitis diagnosed by California mastitis test (CMT) with bacterial isolation from milk samples

	Isolate	No isolate	Total
CMT positive	35	3	38
CMT negative	3	59	62
Total	38	62	100

Two-sided chi-squared (with Yates correction) = 72.5; $p < 0.0001$; OR = 229.4 (95% Confidence Interval = 43.9-1200.0)

Table 3 Frequency of isolation of bacteria from the milk samples in subclinical mastitis of Sahel goats

Bacterial species	Number	%
Staphylococcus	10	26.3*
Streptococcus	4	10.5
Staphococcus and Streptococcus	24	63.2
Escherichia coli	0	0

*Significant ($p < 0.0001$) variation in values (one-way chi-squared test)

DISCUSSION AND CONCLUSION

The results of the study revealed that SCM, based on CMT, in lactating Sahel goats located in Maiduguri (Borno State, northeastern Nigeria) was endemic at a goat-level prevalence of 38%, comparable to the recent prevalence (23-60%) reported in Sahelian goats in other parts (Kaduna, Kebbi and Kaduna States) of northern Nigeria (Udo et al 2019; Danmallam and Pimenov, 2019). The parity of the goats influenced the prevalence without significant trend or association. The primiparous goats had higher prevalence of SCM than those in the 2nd and 3rd parity, but further parity tended to increase the prevalence until 6th parity when the prevalence was beyond primiparous level (75%). This may be related to husbandry practices in the local herds where manual milking of goats for household consumption is rarely done and milk letdown often occurs with the kid suckling the dam. It is presumed that teat injury may be more common in the primipara that is also immunologically naïve to the pathogens associated with SCM, and may not experience spontaneous recovery from teat infections that took place during parturition (McDougall et al, 2002). Parity was similarly reported not to be associated with CMT score (Boscos et al, 1996) and the prevalence of SCM (Hafty et al, 2016; Mishra et al, 2018). However, SCM increased in older dams (Hafty et al, 2016) that were possibly having higher number of parity, and CMT-based prevalence increased with increasing parity (Mahlangu et al, 2018). Parity seemed to have an impact on milk somatic cell count, which could be detected by CMT in goats, with somatic cell count increasing with parity, but the count was higher in primipara than multipara in sheep (Paape et al, 2007).

The SCM was mostly (71.1%, 27/38) mild and rarely severe (10.5%, 4/38) based on CMT score. Severity of SCM was diagnosed by CMT which qualitatively estimated the amount of DNA of inflammatory cells infiltrating the milk in the

mammary gland (McDougall et al, 2010; Persson and Olofsson, 2011; Souza et al, 2012). SCM was significantly associated with intra-mammary bacterial infections which were isolated in the milk samples from 92.1% of cases. The CMT-negative cases with bacterial isolation of staphylococci in milk could be in early infection with undetectable somatic cell count using CMT. The bacteria isolated were either Staphylococcus spp, Streptococcus spp, or both. These infections elicit pro-inflammatory cytokines which cause inflammatory cells to migrate into mammary glandular tissues (Albenzio et al, 2016; Bochniarz et al, 2017; Ruiz-Romero et al, 2020). Most of the reported intra-mammary infections in SCM were staphylococci (Contreras et al, 1995; Marogna et al, 2012; Akter et al, 2020), and they were frequently more of Coagulase-negative than positive strains (Zhao et al, 2015; Dore et al., 2016; Gelasakis et al, 2016). However, this study indicated the involvement of Coagulase-positive strains among 70.6% of the staphylococcal isolates. In northern Nigeria, Coagulase-positive *S. aureus* were reported as the most commonly isolated bacteria from mastitic goats (Ameh and Tari, 1999; Danmallam and Pimenov, 2019). These Coagulase-positive strains cause more losses in milk production than Coagulase-negative stains, and can lead to clinical form with gangrenous lesion (Gelasakis et al., 2016). Additionally, streptococci played contributory role as pathogen of SCM, but *E coli*, an environmental contaminant, was not associated with any case, perhaps due to good hygiene in the herds. Previous reports in the country causally associated SCM with streptococci and *E. coli* (Ameh et al, 1993, 1994; Ameh and Tari, 1999; Danmallam and Pimenov, 2019). These were also bacteria that caused clinical mastitis when the infections persisted in the goat herd.

The major limitation of the prevalence data was the low total number of goats and the low number at strata of parity, especially the higher parity. The goat populations were not large in the area of study, and older goats might have been culled because of unidentified reasons. Nevertheless, this study is considered a pilot non-randomized study to identify the preliminary outlook on SCM existing in the population. As a survey, the study has identified the occurrence of an insidious disease condition which exists without clinical indicators, but could have the potential of decreasing milk yield and quality (Leitner et al, 2004), increasing kid mortality by milk underfeeding and infective enterotoxicity (Contreras et al, 2003; Silanikove et al, 2014), spreading of latent intra-mammary infections that could escalate to clinical mastitis (Koop et al, 2013), and undermining the development of commercial goat dairy production.

In conclusion, SCM of lactating Sahel goats associated with intra-mammary infections by staphylococci and streptococci was prevalent, and the diagnosis of SCM at various levels of parity by CMT was strongly associated with isolation of the bacteria in the milk.

CONTRIBUTIONS

DTY: 1, 2, 3, 4, 5, 6 7, 9 ; DLM: 1, 2, 4, 7, 9; NAI: 7, 8, 9, 10 ; MNJ: 4, 5, 6, 7 ; IOI: 1, 2,3, 4, 6, 7, 8, 9, 10

(1)Conception; (2) Design; (3) Supervision; (4) Fundings; (5) Materials; (6) Data Collection and/or Processing; (7) Analysis and/or Interpretation of the Data; (8) Literature Review; (9) Writing; (10) Critical Review

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CONFLICT OF INTEREST

The authors declared that there was no conflict of interest.

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SUBKLINIČKI MASTITIS SAHEL KOZA U LAKTACIJI U MALIM STADIMA U URBANIM SREDINAMA U MAIDUGURIJU, NIGERIJA: PREVALENCA I INTRAMAMARNE INFEKCIJE

SAŽETAK

Rizik od hroničnog kliničkog mastitisa može biti povećan zahvaljujući prisustvu neprepoznatog subkliničkog mastitisa (SCM) u malim stadima koza u urbanim sredinama. Naše istraživanje predstavlja presječni pregled naizgled zdravih Sahel koza u laktaciji (februar-mart, 2023.) na prevalencu SCM korištenjem California mastitis testa (CMT) i bakterijskih kultura sirovog mlijeka u Maiduguriju, Nigerija.

Pozitivan CMT je označen kao +1 (blagi), +2 (umjereni) ili +3 (teški). Za izolaciju bakterija su primijenjene standardne procedure izolacije kultura. Prevalenca SCM je iznosila 38% (95% CI, 26.9-52.2) na 100 koza, pri čemu je bila ovisna o paritetu (18.7-75.0%) na način da je opadala ($p < 0.05$) s 50.0% u prvom paritetu na 18.7% u trećem, a potom rasla ($p < 0.05$) na 75.0% u šestom paritetu. Paritet nije statistički signifikantno ($P > 0.05$) povezan sa prevalencom. Većina slučajeva (71.1%, 27/38) je bila blaga. Dijagnosticiranje CMT testom je statisitički signifikantno ($p < 0.0001$) povezano s izolacijom bakterija u uzorcima mlijeka. Stopa izolacije je iznosila 92.1% (35/38) u slučajevima SCM-a, s tim da su CMT negativni uzorci mlijeka imali stopu izolacije od 4.8% (3/62). *Staphylococcus spp* i *Streptococcus spp* su kao čista kultura izolirani u 26.3%, odnosno 10.5% od 100 uzoraka. *Staphylococcus spp.* i *Streptococcus spp.* su zajedno izolirani u 63.2% uzoraka. Od ukupno 34 izolata Staphylococcus spp, 24 (70.6%) su bili koagulaza pozitivni. Stopa izolacije stafilokoka (89.5%, 34/38) i streptokoka (73.7%, 28/38) se nisu razlikovale ($\chi 2 = 3.118$; $p < 0.077$). Stoga je neophodno kontrolirati prevalentnu populaciju sa SCM među zdravim Sahel kozama u laktaciji koje imaju intramamarne infekcije sa ciljem prevencije eskalacije do kliničkog mastitisa.

Ključne riječi: Prevalenca, Sahel koza, *Staphylococcus*, *Streptococcus*, subklinički mastitis