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Original Article

Prevalence, antimicrobial resistance of *Staphylococcus aureus* and associated risk factors of camel mastitis in Rayitu district, East Bale, Ethiopia

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Abstract

A cross-sectional study was conducted in the Rayitu district, East Bale zone, Oromia regional state of Ethiopia, from December 2023 to June 2024 to determine the prevalence of *Staphylococcus aureus*, assess the antimicrobial susceptibility profile and associated risk factors of Camel Mastitis. Two hundred lactating camels were examined for clinical and subclinical mastitis using the California Mastitis Test. Bacteriological examination and Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) were used to confirm pathogen identity. Among the four organisms isolated, *Staphylococcus aureus* was involved in a majority of the cases. Antimicrobial susceptibility testing was performed on 35 *Staphylococcus aureus* isolates. The overall prevalence of camel mastitis was 48.5% (97⁄200), with 42% subclinical and 6.5% clinical cases. *Staphylococcus aureus* was the dominant pathogen. Middle Lactation stage and multi parity were significantly associated ($P<0.05$) with mastitis prevalence. The antimicrobial susceptibility test revealed that 57.14% of *Staphylococcus aureus* isolates were resistant to tetracycline. The study highlights that implementing proper milking hygiene, thorough udder and teat washing, and timely treatment of clinically infected camels, are essential strategies to effectively prevent and reduce the incidence of mastitis in lactating camels.

Keywords: antimicrobial, camel, mastitis, prevalence, Rayitu, *Staphylococcus aureus*

1. Introduction

Dromedary camels (*Camelus dromedarius*) serve as multipurpose animals in Ethiopia and provide milk, meat, and transportation. They also act as financial reserves for pastoralists and have important social and cultural significance, contributing to prestige and wealth (Ramet, 2001). Despite their crucial role, camels have historically been overlooked by researchers and development planners in Ethiopia. Interestingly, approximately 60% of the camel population in Africa is found in the Eastern African countries of Sudan, Somalia, Ethiopia, and Kenya, which are important exporters of dromedary camels to the Arabian Peninsula and Egypt (Mirkena *et al*., 2018). According to data from the Central Statistical Agency (CSA) of Ethiopia for 2020/2021, the country is home to an estimated seven million camels.

Camels are the livestock of choice in pastoral areas, producing 1–12 liter of milk daily (Ambel & Tade, 2022), significantly higher than local zebu cattle's daily milk yield. However, research agendas, promotion programs, regular vaccinations, and animal health services have often excluded camels. Consequently, there is limited knowledge about health problems faced by them compared to other livestock (Megersa, 2010). Mastitis is a complex and economically important disease that affects dairy animals worldwide (Ibrahim, 2017; Seifu & Tafesse, 2010). Like other dairy animals, mastitis can affect dromedary camels, with subclinical mastitis being more prevalent than clinical mastitis (Jilo, Galgalo, & Mata, 2017). The key point is that camels are important milk producers in pastoral areas. Still, they have been overlooked in terms of research and health services, leading to a lack of understanding of their health challenges, including the prevalent issue of mastitis.

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According to Seifu and Tafesse (2010), the causative agents of mastitis in dromedary camels have not been identified or thoroughly researched. However, limited available literature indicates that the major bacterial pathogens isolated from subclinical mastitis in camels include *Staphylococcus, Escherichia coli, Corynebacterium, Streptococcus, Bacillus,* and *Micrococcus* species (Hamad, 2012). Furthermore, (Abdurahman, 2006) reported that subclinical mastitis in camels managed under traditional systems is frequently left untreated, often progressing to a chronic condition that leads to permanent loss of milk production.

Multiple epidemiological studies have linked the consumption of non-heat-treated milk and raw milk products as significant contributors to various illnesses (El-Ziney & Al-Turki, 2007; Keba *et al*., 2020). Therefore, the potential zoonotic risks associated with camel's milk should be seriously considered (Abera, Legesse, Mummed, & Urga, 2016). However, in the Oromia region, specifically in the Rayitu district, there is a scarcity of information on the prevalence and characteristics of camel mastitis. In this district, over 90% of the population are pastoralists, and the traditional practice of heat-treating camel milk is taboo. As a result, camel milk is commonly consumed without heat treatment. Milk is often stored and transported at high ambient temperatures after milking. Given these circumstances, there is a pressing need for organized problem-oriented research to monitor the udder health of camels in this region. Such research would enhance the understanding of the prevalence and implications of camel mastitis, ultimately informing strategies to address the potential public health risks associated with the consumption of unpasteurized camel milk.

Although there has historically been a lack of information on the occurrence of mastitis in lactating camels, recent studies have reported the prevalence of this condition in various camel-rearing regions worldwide. These include reports from Somalia (Mohamud, Mohamed, Jama, Mishra, & Mohamed, 2020), Sudan (Alamin, Alqurashi, Elsheikh, & Yasin, 2013), Kenya (Kashongwe, Bebe, Matofari, & Huelsebusch, 2017), Israel (Guliye, Van Creveld, & Yagil, 2002), and different parts of Ethiopia (Abera *et al*., 2016; Geresu, Abera Leliso, & Liben, 2021; Regassa, Golicha, Tesfaye, Abunna, & Megersa, 2013; Seifu & Tafesse, 2010; Wubishet, Dabaso, & Getachew, 2016).

However, there is a paucity of information on the prevalence of camel mastitis, the antibiotic susceptibility profile of *Staphylococcus aureus* (*S.aureus*), and its associated risk factors in the Rayitu district of the East Bale zone in southeastern, Ethiopia. The main objective of this study was to determine the prevalence of *Staphylococcus aureus*, assess the antimicrobial susceptibility profile, and associated risk factors of camel mastitis.

2. Materials and Methods

2.1 Study area

The study was conducted in Rayitu district, which is located in the East Bale zone of southeastern Ethiopia (Figure 1). The district covers an area of approximately 6,139.39 square kilometers. It is bordered by the Ginir district to the north, the Sewena district to the east, the Goro district to the west, and the Somali Regional State to the south. The northern and southern parts of the Rayitu district are dominated by mountainous terrain, while the rest of the district consists of flat, plain land. The district is traversed by several perennial rivers including the Wabi Shebele, Weyib, and Dinikte. In terms of agro-climatic zones, approximately 5% of the Rayitu district is classified as semi-desert, 90% as tropical, and the remaining 5% as sub-tropical.

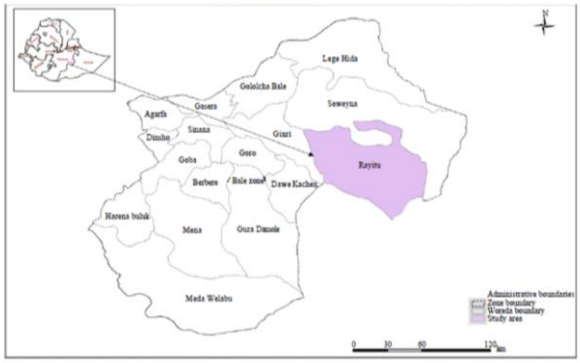

Figure 1. Map of the study area and selected sampling sites

Rayitu District experiences a bimodal rainfall pattern characterized by an erratic distribution. The main rainy season typically extends from March to the end of June, whereas the shorter rainy season usually occurs from September to the end of October. The dominant production system in the district is pastoralist (Yami, Wubie, Cheffo, & Mesfin, 2020). The area is known for its hot, dry climate and is considered a pastoral region. The temperature ranges from mean annual 26°C to a maximum of 40°C, and the average annual rainfall is less than 300 mm. The district lies within an altitude range of 500–1,785 meters above sea level.

2.2 Study population

The study animals were indigenous breeds of the one-humped camel (Camelus dromedarius) that were reared under a pastoral management system. This system allowed camels to graze freely. The camels are known to migrate from areas with feed scarcity to regions with abundant feed, especially during drought seasons. The study population consisted of 200 lactating camels residing in the East Bale, Rayitu district, which were managed under the pastoral production system.

2.3 Study design

A cross-sectional study was conducted to determine the prevalence of camel mastitis, identify the major bacterial pathogens contributing to mastitis, and assess the associated risk factors in the Rayitu district. Management aspects and potential risk factors contributing to the occurrence of mastitis were evaluated by interviewing selected lactating camel owners/herders. The interviewer recorded the responses from the camel owners/herders. The potential risk factors that were given attention during the interviews included age, body condition score, lactation stage (early, middle and late), and the kebele (origin) of the camels. Camel age was estimated using rostral dentition (Bello *et al*., 2013) and categorized as

young (<5 years) and adult (≥5 years). The body condition score of the camels was assessed according to the method described by (Faye, Bengoumi, Cleradin, Tabarani, & Chilliard, 2001) and was grouped as poor (score 1), medium (score 2 and 3), and good (score 4).

To investigate any significant differences in the occurrence of mastitis during these stages, the lactation stage was categorized into three groups: early (1-2 months), middle (3-9 months), and late (10-18 months) according to (Bello *et al*., 2013) This study aimed to determine the prevalence of *Staphylococcus aureus*, assess antimicrobial susceptibility tests, and examine the impact of various risk factors on the occurrence of camel mastitis in the Rayitu district.

2.4 Sample size determination

The desired sample size for this study was calculated using the formula provided by (Thrusfield, 2018). The calculation was performed with a 95% confidence interval (CI) and 5% desired absolute precision. The expected prevalence of 26% was reported by Alebie, Molla, Adugna, Tesfaye, and Ejo (2021) , in the Afar region and served as the reference point for this study. Based on these calculations, the estimated sample size was 296 camels. However, due to the camels' mobility in search of pasture and water during the sample collection period, only 200 samples were ultimately collected in the Rayitu district.

Rayitu district was purposely selected for the study because of its large camel population and accessibility of infrastructure. Six kebeles (the smallest administrative units) were also purposively selected based on their proximity to roads and accessibility to infrastructure. Lactating camels were chosen within each household using a simple random sampling technique. A proportional number of lactating camels from each household were selected for milk sample collection and physical examination.

2.5 Physical examination of the udder

Clinical mastitis was defined according to the criteria outlined by Radostits, Mayhew, and Houston (2000). This includes a swollen, reddened, and hardened udder that is painful upon palpation, along with alterations in the color and consistency of the milk depending on the degree of inflammation. Clinical mastitis was identified by the presence of abnormal milk, signs of udder infection, and positive culture results. In contrast, subclinical mastitis was recognized by apparently normal-looking milk but with an increased leukocyte number, as evidenced by the CMT and a positive culture result, as reported by (Balemi *et al*., 2021).

2.6 California mastitis test

The California Mastitis Test (CMT) was performed before obtaining milk samples for bacteriological culturing. This test was conducted after discarding the first streaks of milk, following which approximately 3 ml of milk per quarter was milked into the CMT paddle, and then a visual assessment of the milk was performed concerning consistency, color, and clots. The milk was then mixed with an equal amount of CMT (3% CMT fluid and blended using a circular motion. The scores were classified into four categories: 0, negative (-) or trace (±); 1, positive (+); 2, positive (++); and 3, positive (+++). Negative (-) and trace (±) reactions were considered **"**negatives**"** and different intensities of positive reactions (+, ++, +++) were considered **"**positives**"** (Seifu & Tafesse, 2010).

2.7 Bacterial isolation and identification

Bacterial isolation and identification were performed according to the Animal Health Institute bacteriology lab-specific Standard Operating Procedures (SOP). Different enrichment media were used, such as buffered peptone water for Salmonella Species identification and Bran Heart Infusion Broth for pre-enrichment of other bacteria. Xylose Lysine Deoxycholate (XLD) Agar, Eosin-methylene blue (EMB) agar, 1% maltose purple agar base, and Edward medium were used as selective media for the isolation of *Salmonella*, *E. coli*, *Staphylococcus aureus*, and *Streptococcus agalactiae*, respectively. **'**For confirmatory tests, a Biolog/Omnilog identification system (OmniLog ID system, Hayward, CA, USA) was used for the identification of Salmonella species, while MALDI-TOF (Bruker, Germany) was employed *for the identification and confirmation of Staphylococcus aureus, E*. coli, and *Streptococcus* agalactiae.

2.8 Antimicrobial susceptibility test

The antimicrobial susceptibility test (AST) was performed on Mueller-Hinton agar using the disk diffusion method (Clinical and Laboratory Standards Institute, 2012), and sheep blood (5 %) was added to Mueller-Hinton agar to test *Streptococcus agalactiae* (*S. agalactiae*). Three to five isolated colonies were transferred to 5 ml of 0.85% saline water. Turbidity was measured using densitometry and was adjusted to 0.5 McFarland. After measuring the turbidity, a sterile cotton swab was dipped into the suspension, and the Mueller-Hinton agar plate was inoculated by rotating at 60°. Antimicrobial discs were applied to the media using a disc dispenser and then incubated aerobically for 16–18 h for both *E. coli* and *S. aureus*, whereas *S. agalactiae* was incubated for 24 h under 5% CO2.

The zone of inhibition was measured using a digital caliper and was interpreted as susceptible, intermediate, or resistant. *S.aureus* tested against penicillin G (10 units), ciprofloxacin (5 µg), cefoxitin, (30 µg), tetracycline (30µg), gentamicin (30 µg), erythromycin (15µg); sulphamethoxazole/ trimethoprim (25 µg), clindamycin (10 µg) (OXOID discs), and *Escherichia coli* (*E. coli*) isolates were tested against ciprofloxacin (5 µg), ceftriaxone(30 µg), tetracycline (30µg), gentamicin (30 µg), ceftazime (30µg); sulphamethoxazole/ trimethoprim (25 µg), ampicillin (10 µg), AMC (30 µg), meropenmem (10 µg) (OXOID discs. Standard breakpoints were interpreted based on Tamma, Harris, Mathers, Wenzler, and Humphries (2023) and *S. aureus* ATCC 25923 was used as the quality control strain for each run.

2.9 Data analysis

The data were fed into MS Excel spreadsheets and analyzed using STATA (MP16.0). The associations of clinical and subclinical mastitis with parity, stage of lactation, tick infestation, lesion, kebele, and herd size were tested with a

chi-square test (χ^2), and $P < 0.05$ is considered statistically significant.

3. Results and Discussion

3.1. Prevalence of camel mastitis at animal and Kebele level based on the CMT in Rayitu district of East Bale zone

From 200 traditionally managed lactating camels examined for mastitis, an overall prevalence of 48.5% (97/200) of clinical and subclinical mastitis was recorded. Of the 97 CMT-positive mastitis cases, 42% (84/200) were subclinical and 6.5% (13/200) were clinical (Table 1).

The highest prevalence of camel mastitis was recorded in Gurara kebele at 11.5%, followed by 9.5% in Halo Choma, 8.5% in Haro Dube, 7.5% in Anole, and 6% in Dacha Bala, with the lowest prevalence observed in Jara Dawe at 5.5% (Table 3).

Table 1. Prevalences of clinical and subclinical mastitis based on the CMT in Rayitu district of East Bale zone

Status	Positive	Prevalence %
Clinical	13	6.5
Subclinical	84	42.0
Total	97	48.5

Table 2. Quarter-level prevalences of Camel mastitis based on the CMT in Rayitu district of East Bale zone

Quarter	Positive	Prevalence %
Right hind	45	5.66
Right left	45	5.66
Left hind	49	6.16
Left front	42	5.28
Total	181	22.77

3.2 Quarter-level prevalence of Camel mastitis

Of the 800 tested quarters, five were blind, and 22.77% (181/795) were found to be positive for subclinical mastitis using CMT. The left hind quarter (6.16%) was the quarter most frequently exposed to mastitis and the left front quarter (5.28%) was the quarter least exposed to mastitis (Table 2).

3.3 Risk factor analysis of lactating camel mastitis prevalence in Rayitu district of East Bale zone

This study found that lactating camel prevalence showed a significant association (P<0.05) with kebeles, parity, and lactation stage, but did not have a significant association (P>0.05) with the age and body condition score of lactating camel (Table 3).

3.4 Bacterial isolation and identification of camel mastitis

Among 97 milk samples subjected to bacteriological examination, 45.36% (44/97) had major mastitis-causing pathogens. Of the major bacterial isolates, 37(84.01%) were *S. aureus*, 4 (9.01%) were *E. coli*, 2(4.55%) were *S. agalactiae* and 1(2.27%) were *Salmonella enterica*. *S. aureus* was the dominant pathogen isolated and *Salmonella enterica* was the least pathogen isolated causing mastitis (Table 4).

Table 3. Risk factors for Camel mastitis based on the CMT in Rayitu district of East Bale zone

Factor	Examined	CMT positive	Prevalence %	Chi^2	P-value
Kebele					
Anolle	16	15	7.5	31.3110	P<0.000
Dacha-Bala	18	12	6		
Gurura	42	23	11.5		
Haro-Dube	30	17	8.5		
Jara-Dawe	48	11	5.5		
Halo-choma	46	41	20.5		
Age					
Young	5	3	1.5	0.2715	P> 0.602
Adult	195	94	47		
Body condition score					
Poor	48	17	8.5	4.3519	P> 0.113
Medium	75	39	19.5		
Good	77	41	20.5		
Lactation stage					
Early	71	28	14	10.1515	P< 0.006
Middle	112	55	27.5		
Late	17	14	7		
Parity					
Single	48	17	8.5	4.3283	P< 0.037
Multi-parity	152	80	40		
Total	200	97	48.5		

Table 4. Major mastitis-causing pathogens identified

Isolated pathogen	Number of isolates	% Total isolates
S. aureus	37	84.01
E. coli	4	9.01
Streptococcus agalactiae	2	4.55
Salmonella enterica	1	2.27
Total	44	100

3.5 Occurrence of antimicrobial resistance in camel mastitis

Among 35 *S. aureus* isolates, 57.14% (20/35) showed resistance to tetracycline, 31.43 % (11/35) were resistant to penicillin, 8.57% (3/35) resistant to cefoxitin, 5.71 (2/35) resistant to erythromycin, and they were susceptible to gentamycin and clindamycin, ciprofloxacin, and sulphamethoxazole/ trimethoprim.

Camel mastitis is a significant disease in traditionally managed lactating camels in the pastoral Rayitu district of the eastern Bale zone. According to the current study, the overall prevalence of camel mastitis was 48.5%. Previous research has reported higher mastitis prevalence rates of 76.4% in Eastern Ethiopia (Seifu & Tafesse, 2010) and 59.8% in Afar (Bekele & Molla, 2001). However, lower prevalence rates of 22.4% in Gomele district of Borena zone (Geresu *et al*., 2021), 32.2% in Jigjiga (Husein, Haftu, Hunde, & Tesfaye, 2013), 44.8% in Yabello district of Borena zone (Regassa *et al*., 2013), and 31% in Gursum district of Eastern Hararghe zone (Mehamud, Megersa, Abebe, & Ahmed, 2017) have also been documented.

The current study reported a prevalence of clinical mastitis at 6.5%, which aligns with the 5.4% rate reported by Regassa *et al*. (2013) in the Yabello district, Borena zone. (Geresu *et al*., 2021) reported a lower prevalence of 4.3% in the Gomole district of the Borena zone. Nonetheless, higher rates of 12.5% were observed in Afar, Ethiopia (Bekele & Molla, 2001) and the Borena zone of the Oromia regional state (Wubishet *et al*., 2016).

At the quarter level, the study found a 5.66% prevalence in the right hind and right front quarters, 6.16% in the left hind quarter, and 5.28% in the left front quarter. These figures are consistent with the quarter-level prevalence reported by (Regassa *et al*., 2013), who found 7.0% in the left hind, 5.1% in the left front, 5.7% in the right hind, and 1.5% in the right front quarters. However, Geresu *et al*.(2021) reported a lower quarter-level mastitis prevalence.

The study identified significant risk factors for camel mastitis, including kebele (administrative unit), lactating stage, and parity. The findings indicated a positive relationship ($p < 0.05$) between mastitis and the middle stage of lactation, which contrasts with the results of Geresu *et al*.(2021) and Regassa *et al*.(2013), who reported a positive association between early lactation stage and camel mastitis incidence. In contrast, age and body condition score were not significantly associated ($P>0.05$) with the prevalence of lactating camel mastitis. Camels in early or peak lactation stages are at higher risk of mastitis due to increased milk production, which can put more strain on the udder (Jilo *et al*., 2017). Camels that have given birth multiple times (multiparous camels) are at a higher risk of developing mastitis because repeated lactation can lead to udder tissue damage. This damage may provide an entry point for pathogens, making these camels more susceptible to infection (Abdurahman, 2006; Jilo *et al*., 2017).

The bacterial analysis of milk samples showed that 84.01% (37/44) of the main mastitis-causing pathogens were *S. aureus*, the most prevalent bacteria. This finding aligns with the higher proportion of *Staphylococcus aureus* (19.8%) reported by Regassa *et al*., (2013). As per Foster & Geoghegan (2024), *S. aureus* can cause long-lasting and severe mastitis because it can inhibit the mammary gland's epithelial cells.

The higher susceptibility of *S. aureus* to antibiotics such as ciprofloxacin, gentamycin, and sulfamethoxazole-trimethoprim suggests that these antibiotics may be more effective in treating camel mastitis caused by *S. aureus* in the study area. The higher resistance to tetracycline compared to penicillin indicates that tetracycline may not be the best first-line antibiotic choice for treating *S. aureus* related camel mastitis in this area, given the prevailing antibiotic usage patterns. Several factors contribute to the variation in camel mastitis prevalence across different regions in Ethiopia. These include management practices and milking techniques (Abdurahman, 2006), parity and age of the camels (Raziq, Younas, Khan, & Iqbal, 2010), environmental conditions and geographic differences (Megersa, 2010), and access to veterinary services (Bekele, 2010).

4. Conclusions

In the present investigation, mastitis emerged as the primary issue affecting lactating camels in the Rayitu districts of the Eastern Bale zone. The bacterial pathogen *S. aureus* was identified as the dominant cause of mastitis in these animals. Factors such as lactation stage, age, and parity were found to be significant predisposing factors for mastitis in lactating camels in the study area. The California mastitis test (CMT), in combination with bacteriological examinations, proved to be highly effective in diagnosing the disease. Implementing proper milking hygiene, thorough udder and teat washing, and timely treatment of clinically infected camels, are essential strategies to effectively prevent and reduce the incidence of mastitis in lactating camels.

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