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Prevalence of Bovine Mastitis, Risk Factors and Isolation of Major Causative Pathogens in and around Hargeisa, Somaliland

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ABSTRACT

A cross-sectional study was conducted from December 2019 to July 2020 on the prevalence of bovine mastitis, risk factors, and isolation of major causative pathogens in and around Hargeisa, Somaliland. The study was carried out on 384 dairy cows. Based on clinical examination, the California mastitis test (CMT), and isolation of bacterial pathogens using standard techniques, the overall prevalence of mastitis was 28%. Among them, 78.7% had subclinical mastitis, whereas 21.3% had clinical mastitis. At quarter level, 414 quarters were examined, and the overall prevalence was 37.7%. The prevalence of blind teats was 2.6% due to the absence of 40 teats during a physical examination. The current study found that the prevalence of mastitis was statistically significant and associated with age, parity, lactation, and previous history of mastitis. On a bacterial examination of the milk sample, the most isolated bacteria was Staphylococcus aureus, which scored 42.5%, while micrococci (13.8%) were the least identified bacteria. The conclusion of this study revealed that there is a high prevalence of mastitis, particularly subclinical mastitis, in the study area. Awareness creation and increasing animal health services, especially regular screening, prevention, and treatment strategies for mastitis, should be needed.

INTRODUCTION

In Somaliland, livestock production is most important sector which is predominantly pastoral and agro pastoral in Somaliland employing over 70% of the population. Its contribute 60% of GPD and around 85% to foreign export earnings (Somaliland National Development Plan, 2012 – 2016). According to FAO, estimate the number of cattle in Somaliland 0.40 million in 2011. How over the milk production does not satisfy the requirement of the country due to magnitude factors. The factors that contribute low milk production in the country include, poor infrastructures, inadequate cold storage facilities, irregular power supply, improper milk handling, and inadequate technology (USAID, 2021) .and poor production due to diseases such as Mastitis.

Mastitis is a complex, multi-etiological illness that is characterized by inflammation of the parenchyma of the mammary glands. It affects both the quality and quantity of milk and is characterized by physical, chemical, and frequently bacterial alterations in milk as well as pathological changes in glandular tissues of the udder (Radostis *et al.*, 2000). Mastitis is a problem that affects all countries, including Somaliland, because it has a negative impact on animal health, milk quality, and the economics of milk production (Sharma *et al.*, 2007).

Apart from the economic losses, mastitis can have serious implications on public health. Mastitis which is mostly caused by the interaction of multiple pathogenic agents (primarily bacteria), can expose human beings to various organisms through infected milk, thus serving as a media for transmission of various zoonotic diseases like tuberculosis, brucellosis, diphtheria, scarlet fever and Q fever (Garvey, 2019). Furthermore, mastitis is a multifactorial disease that is related to the way cows are raised as well as their environment (Sharma *et al.*, 2012). Age, parity, lactation stage, milk supply, breed, genetics, history of mastitis, type of floor, finger and teat disinfection, etc. are all risk factors of this disease (Rahman *et al.*, 2018).

Since there is research gap in the study area about this disease, this study was aimed to assess, Prevalence of Bovine Mastitis, Risk Factors and Isolation of Major Causative Pathogens in and around Hargeisa, Somaliland.

MATERIALS AND METHODS

Study Area and Study Population

A cross-sectional study was conducted from December 2019 to July 2020 on lactating cattle in and around Hargeisa, Somaliland, in order to assess the Prevalence of Bovine Mastitis, Risk Factors and Isolation of Major Causative Pathogens. Hargeisa is the capital city of Somaliland. It is located in an enclosed valley of the Galgodon (Ogo) highlands, at an elevation of 4,377 feet (1,334 metres). The population of Hargeisa was estimated at 1.2 million in 2019, and it also has a hot, semi-arid climate with temperature ranges of 18 to 24 °C. Furthermore, the annual rainfall of the city ranges from 400 mm to 600 mm. The target population was all lactating zebu cows and crossed breed cows in and around Hargeisa.

Sampling Method and Sample Size

Simple random sampling is used to sample local indigenous lactating dairy cows in and around Hargeisa.

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The formula provided by Thrusfield (2018) was used to compute the required sample size, taking into account the desired absolute precision of 5%, the 95% confidence level, and the 50% predicted prevalence due to the lack of previous research in the area. So, a total of 384 dairy cows were selected and employed in the study.

$n = 1.962 * Pexp (1-Pexp)/d2$

Where.

n required sample size

P expected prevalence

d^2 desired absolute precision

Study Methodology

Physical Examination

Briefly, udders or teats were physically examined first by visualization and then by palpation to detect the presence of gross lesion. During examination attention was paid to cardinal signs of inflammation, symmetry, size and consistency of udder quarters. Clinical mastitis was diagnosed on the basis of manifestation of visible signs of inflammation and abnormal milk. At the time of each examination, the breed of the cow, age of the cow, health status of the mammary glands and the respective farm names were recorded.

California Mastitis Test

In accordance with the National Mastitis Council recommendations, with a little change, milk samples were gathered to detect subclinical mastitis. A significant amount of dirt needed to be cleaned; therefore, the udder was briefly washed with tap water and dried. Cotton soaked in 70% ethyl alcohol was used to wipe the ends of the teats. 5 to 10 ml of milk were aseptically collected from each quarter of the lactating cattle into a pedal, and then equal amounts of the California Mastitis Test (CMT) reagent and sampled milk were added to each segment of the CMT paddle and gently circled for 15 seconds.

Based on the thickness of the gel created by the CMT reagent and milk mixture, the test result was read as ranging from 0 (the mixture remained unchanged) to 3 (a nearly solid gel formed), with a score of 2 or 3 being regarded as a positive result (AHDB, 2019). When at least one-quarter of the cows tested positive for CMT, the cow was termed positive.

Milk Sample Collection

A standard milk sampling method was used to gather the milk samples. The udder was cleaned with tap water and dried. Cotton that had been soaked in 70% ethyl alcohol was used to wipe the teat ends. After removing the first three milking streams, 10 ml of milk was collected in a sterile test tube. The collection date, household, and sample code were then clearly written on each sample using a permanent marker. The samples were taken to the Ministry of Livestock and Fisheries laboratory in Hargeisa after being collected and put in an icebox.

Bacterial Isolation and Identification

Bacteriological testing was conducted after the collection of a positive milk sample in accordance with NMC and national committee for clinical laboratory standards. The quadrant streaking technique was used to streak a single standard loop of milk sample on 7% sheep blood agar and MacConkey agar for every cow. Bacterial growth was assessed after an aerobic incubation period of 24 to 48 hours at 37°C. After growth, isolated bacteria were first identified using colony morphological characteristics, hemolytic reactions (in initial cultures), and gram staining reactions (bacterium shape and arrangement). To obtain pure colonies these colonies were subculture to nutrient agar, MacConkey agar, Edwards medium and biochemical tests including catalase, oxidase, coagulase, sugar fermentation test, oxidation fermentation test, and indole test.

Data Management and Analysis

The collected data were entered into the Microsoft Excel programed and analyzed using SPSS statistical software version 21. Descriptive statistics were used to summarize data and were presented in the form of frequencies, percentages, and tables. Odds ratios by logistic regression were used to reveal the presence of an association between potential risk factors and the occurrence of mastitis. All statistical tests were considered significant at $p < 0.05$.

RESULT

Prevalence of Bovine Mastitis

According to a clinical examination and CMT test, 28% (108/384) of the 384 lactating dairy cows evaluated

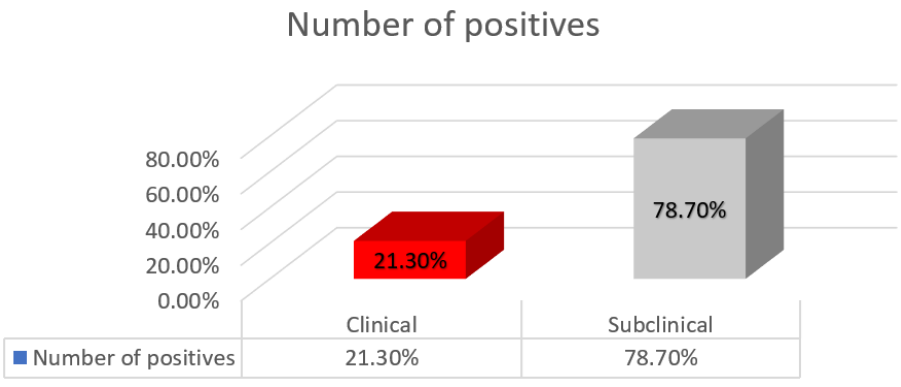

Figure 1: Prevalence of clinical and sub clinical mastitis at cow level

had mastitis overall. Among them, 78.7% (85/108) had subclinical mastitis, whereas 21.3% (23/108) had clinical mastitis.

Prevalence of Bovine Mastitis at Quarter Level

Based on physical examination and CMT, the quarter-level prevalence of mastitis was 37.7% (414/1098). In comparison to prevalence of Right hind (31.2 %) and Left hind (28.7%) was higher than the prevalence of left front (21.3%) and Right front (18.8%). The prevalence of blind teats was 2.6% overall during physical examination due to the absence of 40 teats.

Table 1: Prevalence of clinical and sub clinical mastitis at quarter level

Quarter teat	NO. Negative	No. Positive	Prevalence	Number of blind teats
Right front	22O	78	18.8%	9
Right hind	300	129	31.2%	13
Left front	325	88	21.3%	7
Left hind	253	119	28.7 %	11
Total	**1098**	**414**	**100 %**	**40**

Bacterial Isolation in the Study Area

Milk samples of 414 quarters, which were positive with CMT and clinical examination were made bacterial culture in laboratory. The major bacterial isolate and their prevalence showed by table 2, the predominantly bacteria which had greater prevalence were Staphylococcus aureus scored 42.5%, followed by Streptococci (24.9%), E. coli (18.8%) and Micrococci was the least isolate which accounts for 13.8 %.

Table. 2: Bacteria species isolated from clinical and sub clinical mastitic cows

Bacteria species	Number of isolate	Prevalence
Staphylococcus aureus	176	42.5%
Streptococci	103	24.9%
Micrococci	57	13.8%
E. coli	78	18.8%
Total	**414**	**100 %**

Associated Risk Factors of Bovine Mastitis in Study Area

This study also evaluated the major risk factors associated bovine mastitis in study area. The below table showed all of them: The analytic result revealed that the lactating cows older than 9 years (49.1%) had more prevalent than those aged less than 9 years (28.7%, 22.2%).

During the study lactation stage of cow's exanimated, and result indicated that cows in early stage of lactation had greater prevalence of mastitis 55.6 % than those in middle and late stages (17.6% and 26.8%) respectively. It was also showed that there was association between mastitis and lactation stages because it was statistical significance among them (P<0.05).

In the study location, cows with eight parities or more had a higher mastitis prevalence rate, scoring 55.8% compared to cows with four to seven parities or less, which scored 30.6% and 26%, respectively. The study was statistically significant for group variation (P 0.05).

A score of 72.2% and 27.8%), respectively, indicated that cows with a prior history of mastitis had a higher prevalence of this disease than cows without a previous history. Between the two groups, there was a statistically significant difference in mastitis prevalence (P < 0.05).

Table 3: Analysis result of risk factors for the occurrence of bovine mastitis in the study area

Risk factors	Total No. of animals examined	Prevalence	Crude OR & (95) CI	Adjacent OR & (95) CI	(P-value)
Age					
3-5 (young)	167	22.2%	0.115-0.381	0.097 -0.448	0.000
6-9 (adults)	116	28.7%	0.262-0.801	0.412- 1.892	0.000
>9	101	49.1%	*	*	0.749
Lactation					
Early	142	55.6 %	1.515-4.534	1.439- 5.989	0.000
Middle	138	17.6 %	0.144-0.609	0.166- 0.919	0.003
Late	104	26.8 %	*	*	0.031

Parity					
1-4	142	26%	0.115-0.352	0.074- 0.323	0.000
4-8	131	30.6	0.75-0.257	0.063- 0.325	0.000
>8	111	45.4%	*	*	0.000
Previous History					
Yes	123	72.2%	6.455- 18.055	6.453- 24.185	0.000
No	261	27.8%	*	*	0.000

*Note: *(statistically significant)*

DISCUSSION

The overall prevalence of bovine mastitis in Hargeisa was 28% (108/384). The current result was greater than the prior prevalence in Tanzania (21.7%) reported by (Mdegela *et al.*, 2009) and (Tolosa *et al.* 2009), who demonstrated a frequency of 27.3% in South Eastern Ethiopia. The results of the study support those of Bitew *et al.* (2010), who found a prevalence of 28.2% in Bahir Dar and its surroundings. Additionally, this finding was lower than the prior prevalence of bovine mastitis recognized by (Seid *et al.*, 2010), who found 37.2% in urban and rural dairy farms in Addis Abeba. However, it is significantly lower than OYOO (2017), who reported a prevalence of 50.7% overall in Kenya. The variations in geographic location, management practices, the breed of cattle under investigation, and environmental factors can help to explain why those outcomes were different.

Furthermore, the average prevalence of clinical mastitis in the study area was 21.3%. Current findings agree with precious bovine clinical mastitis (21.2%) stated by (Tilahun and Aylate, 2015) in Addis Ababa, Ethiopia. This study was higher than the prevalence of clinical mastitis (6.8%) discovered by Mbindyo *et al.* (2022) in Kenya. However, the prevalence of clinical mastitis in Hargeisa was higher than the prior result determined by Telosa *et al.* (2009), who reported a prevalence of 9.5% at Wolayta Sodo, Ethiopia.

The overall prevalence of subclinical mastitis recorded in the study area was 78.7 %. Based on these findings, the result was higher than the past prevalence of 36.6% determined by Sori *et al.* (2005) in and around Sebeta, Ethiopia; 68.6% stated by Miyama *et al.* (2020) in Uganda; and Kamirimuribo *et al.* (2008) who estimated the prevalence of sub clinical mastitis (75.9%) in Tanzania. The high prevalence of sub-clinical mastitis in the study area is due to low hygiene before and after milking, a lack of hand washing before milking, dry cow management, and inappropriate therapy for mastitis during lactation. Furthermore. The invisible clinical sign of sub-clinical mastitis secretes apparently normal milk, which draws little attention from farmers.

Based on CMT and the physical examination of quarters, the overall prevalence was 37.7% in the study area. The current result was higher than the prevalence of 30.32% reported by Biniam *et al.* (2015) in Dire Dawa town, Ethiopia, and Bachaya *et al.* (2011), who reported the prevalence of 35.25% in Pakistan. Right hind had a higher prevalence, followed by left hind. This finding agrees with the previous result shown by (Bayush and Abera, 2018). This might be due to the high production capacity of the hind quarters (Radostits *et al.*, 2007) and the high contamination of environmental pathogens.

The study investigated major bacterial pathogens in mastitic milk from positive cows in the study area. The predominant bacteria were Staphylococcus aureus (42.5%), followed by Streptococci (24.9%), E. coli (18.8%), and Micrococci (13.8%). The bacteria that caused the most cattle mastitis in the study area was S. aureus bacteria, and these findings agreed with the 42.6% determined by Belay *et al.* (2021) in Ethiopia. However, the analytic result was lower than the previous finding (49%) in Pakistan, as stated by (Khan and Muhammad, 2005) and Gitau *et al.* (2014) at 72.9% in South Africa.

According to other research (Mekbib *et al.*, 2010; Atyabi *et al.*, 2006), the second causative agent in the research area was Streptococci spp., and this study is in accordance with the previous result of 26.2%. (Belay *et al.*, 2021) and Kube *et al.* (2020) who reported 21.29%. On the other hand, this finding was lower than 44.1% (Abed *et al.*, 2021) in Egypt. The third causative agent was Escherichia coli, and this result was higher than the 13.31% reported by Tesfaye and Abera (2018) in Jimma, Ethiopia, and the 13.8% reported in Kenya by Ndirangu *et al.* (2022). The current result also agrees with the 17% determined by Ameen *et al.*, (2019) in Egypt.

The last causative agent in the research area was micrococci. The current report was lower than the previous result determined by Belachew (2016) in Ethiopia. On the other hand, it is higher than 6.3% in Tanzania. Most causative agents in the study area are environmental pathogens, and this clear Lack of dry cow therapy, post-milking teat disinfection, lack of hand washing before milking, and poor household hygiene were all factors in the high prevalence of mastitis in the study area.

This study also investigated the relationship between bovine mastitis and associated risk factors in the research area, as shown below. The study found that the prevalence of mastitis was statistically significantly correlated with age (P 0.05), with older cows being more at risk. Age was one of the predisposing factors, according to the examination of the purported risk factors. The current study's observation of increased mastitis cases in older animals lines up with reports made by Biffa *et al.* (2005) in southern Ethiopia. Blowey and Edmondson (2010)

also noted that older cows were more likely to develop mastitis than young and adult animals. Mastitis develops in older cows because they have been milked more frequently, causing the canal to sustain more damage, become dilated, and remain partially open. This makes it easier for skin and environmental pathogens to colonise the udder and produce mastitis.

Mastitis incidence and lactation stage had a statistically significant relationship (P 0.05). The findings indicated that early lactation was more common than late lactation. The fact that there are more occurrences at an earlier period of lactation may be because dry cow therapy is not available in London (Quinn *et al*., 2005). In a similar vein, studies by (Kerro and Tareke, 2003) in southern Ethiopia and (Radostits, 2003) in London suggested that the mammary gland is more vulnerable to new infections during early lactation and the late dry period. This may be because udder washing and teat dipping are not performed during these times, which may increase the number of potential pathogens on the skin of the teat.

Due to their significance (P 0.05), the current study was compared to the earlier publications (Biffa *et al*., 2005; Mekibib *et al*., 2010; Haftu *et al*., 2012) and demonstrated that the occurrence of mastitis has a link with parity. This may be because there is a greater chance of infection over time and a longer infection period, particularly in a herd lacking a mastitis management program and due to an increase in teat injuries (Radostits *et al*., 2007).

Cows with a history of mastitis are more likely to have mastitis in the study area. Furthermore, it displayed greater significance (P 0.05). Researchers from Ethiopia, India, and Brazil, including Mekonnen *et al.* (2017), Kumar *et al.* (2016), and Oliveira *et al.* (2015), reported on a similar conclusion. This could be the result of inadequate follow-up of clinical and chronic mastitis in the research area, a lack of screening tests and treatments for subclinical mastitis, and an extended period of time during which the organism remained in the breast tissue, according to Biffa (1994).

CONCLUSSION

The results of the current study indicated that the study area had a high prevalence of bovine mastitis, particularly the subclinical form. The primary causes of the rise in mastitis in dairy cows in the study area were poor sanitary management, in particular, an unclean environment, a lack of hand-washing and udder cleaning, insufficient preventive and control measures, and a prevalence of the risk variables indicated. The key pathogens identified by this investigation as being responsible for the disease were Staphylococcus aureus, streptococci, micrococci, and E. coli, all of which were isolated from milk samples that tested positive. Since all pathogens are zoonotic agents, as we've shown in the current results, consuming raw milk and its byproducts poses a bigger risk to the public's health.

Therefore, the current study recommends, application of a feasible mastitis intervention plan is required, with a focus on sub-clinical mastitis in particular. These include creating awareness for the population and enhancing hygiene milking practices. On the other hand, the government should increase animal health services, particularly regular screening of dairy cows for subclinical mastitis, treatment of cases both during lactation and during the dry period, and culling of chronically infected animals. Future researchers must make a deep screening of all zoonotic diseases from mastitis milk since there is a gap in the study area about these diseases.

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