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A Cross-Sectional Study on the Prevalence of Mastitis and Associated Bacterial Pathogens in One-Humped Camels (*Camelus dromedarius*) in Pastoral Area of Borena Lowland, Southern Ethiopia

Tesfaheywet Zeryehun, Godana Haro and Bedane Adane

College of Veterinary Medicine, Haramaya University, P.O. Box: 138, Dire Dawa, Ethiopia

Abstract: This study was carried from December 2015 to April 2016 to estimate the prevalence of mastitis, to assess the associated risk factors, and to isolate the major bacterial pathogens in camels in Borena lowland of southern Ethiopia. A total of 1392 quarter milk samples from 348 traditionally managed lactating camels (*Camelus dromedarius*) in which udder was carefully inspected were examined using California mastitis test (CMT) as screening test and standard bacteriological methods were used to isolate and identify the bacterial pathogens. Clinical mastitis was diagnosed in 5.7% animals and 25.7% quarter milk samples were CMT positive. Additionally, 31 (2.2%) teats were blind. Only 37 quarter samples positive for CMT were negative for bacterial growth. The major mastitis pathogens isolated included species of Staphylococcus, and Streptococcus mainly, but also *Micrococcus*, *Bacillus*, *Escherichia coli*, *Klebsiella* spp. and other *Enterobacteriaceae*. The mastitis (clinical and subclinical) prevalence has been significantly increased with age ($p<0.01$), parity number ($p<0.001$), lactation stage ($p<0.001$) and poor body condition score ($p<0.001$). Moreover, tick infestation, udder lesions and deformities were also significantly associated with mastitis. These results demonstrate that clinical and subclinical mastitis is a major problem in traditionally managed camels and hence warrants serious attention.

Key words: Bacterial Pathogens · Borena · California Mastitis Test · *Camelus dromedarius* · Ethiopia · Mastitis · Prevalence

INTRODUCTION

Camels (*Camelus dromedarius*) are vital domestic animal species that are best adapted to harsh environment and fluctuating nutritional conditions of arid, semi-arid and extreme arid zones. These animals are endowed with extra-ordinary features that enable them to survive and perform in such hard conditions [1]. Dromedaries are versatile living assets that ensure food security even during the dry periods and also serve as means of transportation in most inhospitable areas of the world, represent an important security for movement and drought power [1, 2]. Africa hosts 80% of the world population of dromedaries (16.5 millions) of which 63% attributed to East Africa [3].

Camels are the subset of huge livestock resources in Ethiopia with the population estimated to be over one million. The arid and semi-arid areas of the country that contribute more than 60% of the total area and regroup 7.5 million pastoral and agro-pastoral communities [4] are suitable for camel production. The eastern and southern parts of the country, namely Afar, Somali and Borena are the major areas where camel husbandry is widely practiced. In these areas, the livelihoods of the pastoral communities are certainly insured by dromedaries [5, 6].

Camels are important milk producers in arid lands and camel milk is an essential food for livelihood of people and it may be the only milk available in place where other milk producing animals cannot be maintained. The Borena pastoralists, who traditionally are based on cattle husbandry for milk production and wealth storage, have recently developed considerable interest in camel production. Camel milk is popular among pastoralists and has been steadily gained popularities among urban dwellers in many countries [5]. Camel pastoralists prefer camel milk to other types of milk due to the fact that it is

Corresponding Author: Tesfaheywet Zeryehun, College of Veterinary Medicine, Haramaya University, P.O.Box 138, Dire Dawa, Ethiopia.

nutritious, thirst quenching, easily digestible and possesses superior keeping quality to cow's milk due to its high contents of proteins that have inhibitory properties against bacteria that makes raw camel milk a marketable commodity, even under condition of high temperatures and very basic hygiene [6].

Diseases of camels are among the factors that hamper production. Several diseases which directly or indirectly lead to reduce productivity through reduced production and death have been reported in this animal species [1, 6 & 7]. In addition to that, the lack of appropriate traditional knowledge of husbandry practices, labour input requirements for its management and knowledge of its peculiar biology like inducible ovulation are perhaps the possible constraints of camel production in the area [8].

Very little work has been done on udder health in general and mastitis in particular, in camels as the disease thought to be uncommon in this specie comparing to studies on sheep and cows. Today, however, cases of mastitis in camels have been reported from a number of camel keeping countries including Ethiopia [1, 9 & 11-13]. There are few studies concerning aetiology, occurrence and pathogenesis of mastitis in camelidae [9]. Many different bacteria have been isolated from mastitic mammary glands in camels either in the form of pure or mixed infection [9, 13& 14].

Despite its ecological and prestige importance in addition to serve as a source of milk, meat, transportation and income generation for Borena pastoralists, until recently the animals were neglected [11]. Research agendas, promotion programs, regular vaccination and animal health service deliveries are almost always excluding camels. Information on camel mastitis is poorly documented too but has recently received more veterinary attention as a disease of camels. One of the prevalence rates of camel mastitis documented so far in this area is 12.98% by Bekele [15]. The above finding indicated us the danger of mastitis milk for health of consumers and their calves which warrant continuous survey to manage the infection consistently. Therefore, the objectives of this study were to determine the prevalence of mastitis in camels in the establish relationship between predisposing risk factors and the prevalence of mastitis in camels and isolate major bacterial species responsible for mastitis in Yabello district.

MATERIALS AND METHODS

Study Area Description: The study was conducted from December 2014 to April 2015 in the Borena lowland in selected pastoral association such as Surupa, Haro-bake, Darito and Gagna. Yabello is an administrative seat of Borena zone. This lowland area is endowed with, approximately, about more than 35, 000 human populations. Due to existing veterinary facilities, Yabello town was selected as the centre of this study. The Yabello town is geographically located 5°23^{l}49N and 39°31^{l}52E, at a distance of 565 km South of Addis Ababa. In the study area, animal husbandry is generally predominated by extensive pastoral production system. Cattle (232 949) are dominating animal species followed by goats (98 781), sheep (39 073) and camels (22 972) [16].

Study Population: The required sample size for this study was established by taking 12.98% as a previously reported mastitis prevalence rate [15]. Sample size was determined using the formula for cluster sampling [17]: $N = 1.96^2 x\ P_{exp}\ x\ (1-P_{exp})/d^2$ where N was the total number of animals to be included in a sample size, d the desired precision and P_{exp} the expected prevalence. Based on the above formula a minimum of 174 lactating camels was required to be sampled and for precision this number can be inflated twice [17], consequently the sample size of lactating camels was 348.

Protocol Design and Method: Cross-sectional study was conducted on 348 traditionally managed lactating camels around Yabello district. The camels were from four villages namely, Surupa, Haro-bake, Darito and Gagna. The village were conveniently selected and studied, depending on accessibility and willingness of the owners. Sampling animals were made purposively on lactating camels. Before starting the present study, pre-testing survey was made to avoid or minimize ambiguity or challenge that would happen and to adjust the sampling procedure in such a way that it did not disrupt the routine work of herders. Clinical examination and milk sample collection was carried out early in the morning at villages and during midday at watering points and when camels did not show active grazing or browsing and they were under shade. Information on each animal such as age, parity and lactation stage and body condition were taken into account and recorded during sample collection. Calves were allowed to suckle for short time, prior to milking to stimulate milk let-down. Milk samples were collected aseptically after the teat ends were cleaned and rubbed with cotton moistened in 70% ethanol alcohol. Then milk sample was collected in sterile test tube. Sample was labelled and packed in ice-box before transport to Yabello Veterinary Regional Laboratory or Yabello Pastoral and Dry Land Agriculture Research Center Animal Health Laboratory for bacteriological examination.

Camels included in the study were individually identified and clinically examined. During clinical examination, udder abnormalities such as swelling, presence of lesion, udder asymmetry or anatomical malformations were recorded. The size of the rear and forequarters, indurations and fibrosis were examined by deep digital palpation. Tick infestation and the use of anti suckling device were also noted. The milk was examined for its consistency, colour and other visible abnormalities. Clinical mastitis was recognized by outward signs of pathological udder such as swelling, pain, redness, heat and presence of red blood cell in acute mastitis, whereas pathological changes of udder such as hardening, blockage of teat, atrophy or fibrosis and abscess formation were recognized as chronic mastitis and subclinical mastitis were recognized by apparently normal milk coupled to an increase in leukocyte counts as evidenced by CMT (California Mastitis Test) and positive bacterial cultures.

California Mastitis Test (CMT) was carried out using the method described by Schalm *et al.* [18]. The CMT reaction was graded as 0, trace (T), 1+, 2+ and 3+, thus forming five category classes. CMT score of 0 was considered as negative while CMT scores as trace, 1+, 2+, or 3+ were considered as indicators of subclinical mastitis. Microbial examination of the milk sample was performed serially to identify major bacterial agents associated with mastitis in camels. Bacteriological examinations of CMT positive milks were carried out following standard methods [19, 20].

Statistical Analysis: Data entry was performed using Microsoft Office Excel and processed using SPSS version 15th Statistical Software after importing the data from Microsoft Excels. Descriptive statistics were also computed for all the parameters. Chi-square (Fischer's exact test) was used to analyse the differences in positivity between ages, parities, lactation stages, tick infestation, body condition, udder lesions and deformities. A p-value less than 0.05 was considered to be statistically significant [21].

RESULTS

In this study, a total of 348 lactating camels were examined clinically as well as sub-clinically using CMT with subsequent bacteriological examination. Clinical and subclinical mastitis were prevalent in 20 (5.7%) and 156 (44.8%) animals, respectively, leading to an overall mastitis prevalence of 50.6% at the animal level (Table 1) and 25.6% at the quarter level (Table 2). Out of the total examined animals, 21 (6.0%) had one blind teat, while 5 (1.4%) had only two functional teats. Taking clinical mastitis (20) and blind teats (26) into account, 46 camels (13.2%) had no four quarters for milk production. As shown in Tables 1 and 2, the highest mastitis prevalence at the cow level and at the quarter level was observed in Darito while the lowest prevalence was recorded in Haro-bake and in Surupa. Specifically, a high frequency of subclinical mastitis was found in the Darito pastoral association (27.00%) compared to the other pastoral associations.

Prevalence of subclinical mastitis with respect to some exposure variables such as tick infestation, various udder lesions and udder deformities in 348 examined lactating camels were 71 (49.3%), 9 (90%) and 15 (93.8%) respectively as indicated in Table 3.

The associations between the subclinical mastitis prevalence and the age, parity, stages of lactation and body condition score were investigated (Table 4). The prevalence of subclinical mastitis in camels has increased with the age and was highest in 11-15 years old animals. Significantly higher ($p< 0.001$) mastitis prevalence was recorded in camels with parity > 3: among the 152 CMT positive animals, 88.9% of them was found in 7-10 parity and 49.6% in 4-6 parity. In the same way, the frequency of subclinical mastitis was gradually and significantly increased with the lactation stage ($p < 0.001$), 84.6% of camels with 15-20 months lactation stage have exhibited a positive CMT. In addition, subclinical mastitis was also detected significantly more often (in 71.7% of them, $p < 0.001$) in camels in poor condition then in those with medium or good condition.

Among the 306 CMT positive cultured milk samples, 265 (87.7%), yielded bacterial growth, while growth was not observed in 37 (12.3%) of them. A total of 26 (8.6%) quarter milk samples positive for bacterial culture were contaminated by at least 2 more different pathogens based on colony characterisation and Gram's stain. As reported in Table 5, 219 bacteria were isolated and identified from culture positive samples. Gram positive *cocci* were the most dominant bacteria comprising 72.6% of the isolates. More specifically, *Staphylococcus* species (39.3%), *Streptococcus* species (28.8%), *E. coli* (9.6%) and *Bacillus* species (9.1%) were the major isolates.

Table 1: Mastitis prevalence and frequency of teat anomalies in camels (n = 348) in Yabello district, Borena zone

	Surupa (n = 112)	Haro-Bake (n = 96)	Gagna (n = 65)	Darito (n = 75)	Total (n = 348)
Camels with blind teats					
1 blind teat	4	4	1	12	21
2 blind teats	1	1	1	2	5
3 blind teat	0	0	0	0	0
4 blind teat	0	0	0	0	0
Total	5	5	2	14	26
Camels with mastitis					
Clinical cases	9	7	1	3	20 (5.7%)
Sub-clinical cases	36[a]	28[a]	43[ab]	49[b]	156 (44.8%)
Total	45	35	44	52	176 (50.6%)

Different superscripts a, b in the same row indicate significant differences ($p < 0.05$ or more) between pastoral associations.

Table 2: Mastitis prevalence and frequency of teat anomalies according to the udder quarters (n = 1392) of camels in Yabello district, Borena zone.

	Surupa (n = 448)	Haro-Bake (n = 384)	Gagna (n = 260)	Darito (n = 300)	Total (n = 1392)
Blind teats	6 (1.34%)	6 (1.34%)	3 (1.15%)	16 (5.33%)	31 (2.23%)
Udder quarters with mastitis					
Clinical cases	9 (2.00%)	7 (1.56%)	1 (0.38%)	3 (1.00%)	20 (1.44%)
Subclinical cases (CMT > 0)*	88 (19.64%)[a]	75 (19.53%)[a]	62 (23.85%)[ab]	81 (27.00%)[b]	306 (21.98%)
Trace	19 (4.24%)	18 (4.69%)	15 (5.77%)	26 (8.67%)	78 (5.60%)
1+	9 (2.00%)	4 (1.04%)	5 (1.92%)	9 (3.00%)	27 (1.94%)
2+	7 (1.56%)	3 (0.78%)	1 (0.38%)	3 (1.00%)	14 (1.01%)
3+	53 (11.83%)	50 (13.02%)	41 (15.77%)	43 (14.33%)	187 (13.43%)
Total	103 (22.99%)	88 (22.92%)	66 (25.38%)	100 (33.33%)	357 (25.65%)

CMT: California mastitis test

*Total subclinical cases

Different superscripts a, b in the same row indicate significant differences ($p < 0.05$ or more) between pastoral associations

Table 3: Prevalence of subclinical mastitis based on exposure variables in camels (n = 348) in Yabello district, Borena zone

Exposure variables	Number examined	Subclinical mastitis	Prevalence (%)
Tick infestation	144	71	49.3
Udder lesions[a]	10	9	90.0
Udder deformities[b]	16	15	93.8

[1]Udder lesions refer any type of wound on udder and teats; [2]Udder deformities refer any deviation of udder and teats from normal shape and size.

Table 4: Prevalence of subclinical mastitis based on age, parity, lactation stage and body condition score in camels (n = 348) in Yabello district, Borena zone.

	Subclinical mastitis		
Variables (RF) No.exami	Number	Prevalence (%)	p value
Age (years)			
5-10 (n = 233)	87	37.34	< 0.01
11-15 (n = 5)	4	80.00	
15-20 (n = 110)	61	55.45	
Parity (unit?)			
1-3 (n = 200)	68	34.00	<0.001
4-6 (n = 121)	60	49.59	
7-10 (n = 27)	24	88.89	
Lactation stage (months)			
1-7 (n = 204)	76	37.25	<0.001
8-14 (n = 118)	54	45.76	
15-20 (n = 26)	22	84.62	
Body condition			
Good (n = 80)	36	45.00	<0.001
Medium (n = 215)	78	36.28	
Poor (n = 53)	38	71.70	

Table 5: Pathogens isolated and identified from mastitis (clinical and subclinical)-affected quarter milk samples positive for bacterial cultures in camels (n = 348) from the Yabello district, Borena zone

	Number of cases		
Mastitis pathogens	Clinical mastitis	Subclinical mastitis	Total number (%)
Staphylococcus hyicus	1	21	22 (10.0)
Bacillus spp.	1	19	20 (9.1)
Staphylococcus	2	35	37 (16.9)
Streptococcus agalactiae	3	18	21 (9.6)
Staphylococcus intermedius	1	11	12 (5.5)
Micrococcus spp.	1	9	10 (4.6)
Streptococcus dysgalactiae	1	28	29 (13.2)
Streptococcus uberis	1	12	13 (5.9)
Escherichia coli	5	16	21 (9.6)
DNase negative *Staphylococcus*	0	15	15 (6.8)
Other *Entrobacteriaceae*	0	14	14 (6.4)
Klebsiella	0	5	5 (2.3)
Total	16	203	219 (100)

DISCUSSION

Camels are still multipurpose animals increasingly kept for milk, but specialization on the way [22]. The number of reports of mastitis in traditionally managed camels is increasing and likely continues to rise as the milk production per individual camel gradually increases. Accordingly, the clinical mastitis prevalence of 5.7% in this study is higher than that (2.1%) reported by Woubit *et al.* [13] from Southern Ethiopia but, it is lower than prevalence of 12.5% reported by Husein *et al.* [12] from Eastern Ethiopia but much lower than the prevalence (19.5%) found in Eastern Sudan by Wanjohi *et al.* [23]. The prevalence of sub-clinical mastitis per quarter (21.9%) observed in the present study is comparable to the reports of Almaw and Molla [10] (20.5%) at the quarter level using CMT test, Nevertheless, Husein *et al.* [12] reported a relatively higher (25.3%) of subclinical mastitis from Eastern Ethiopia compared to the present study.

The present study also revealed that 31(2.2%) of 348 animals at quarter levels have no sound teat for milk production, suggesting the considerable economic losses due to mastitis and similarly Obied *et al.* [23] reported that only 5% of milking camels in Eastern Sudan had sound and health udder. The rate of milk samples reacting positively with CMT but yielding to no bacterial growth is lower (12.3%) than that of Abdurahman and Younan [22], who reported that 43% of CMT-positive quarter milk samples of camels did not show any bacterial growth. Indeed, milk samples which have been collected in the convalescent phase of infection may react positively upon CMT analysis due to a high leukocyte count during this phase, but may lead to no bacterial growth because of disappearance of any bacteria; specifically, *Escherichia coli* and other Coliforms are known to be rapidly destroyed by inflammatory reactions. It has also been reported that 57% of Coliform infections usually last less than 10 days and milk samples can be considered as culture negative in 20% of clinical cases of mastitis [18, 20]. In addition, milk samples from cows with clinical mastitis or from cows whose milk has high somatic cell counts often yield to no organism on culture [24]. In other cases, the infection may have been eliminated, but an elevated SCC may persist because of infiltration of the udder tissues.

The bacteria isolated from camel milk samples in the present study were *Staphylococcus* species (39.3%), *Streptococcus* species (28.8%), *E. coli* (9.6%), *Bacillus* species (9.1%), other *Enterobacteriaceae* (6.4%), *Micrococcus* species (4.6%) and *Klebsiella* (2.3%) were known as pathogens causing mastitis in cows and goats [25]. Gram-positive cocci, mainly *Staphylococcus* and *Streptococcus* species, were the most dominant udder pathogens isolated in the present study and were also regarded as important mastitis pathogens in camels by other authors [13, 23, & 26]. Younan *et al.* [27] documented that *Staphylococcus aureus* and other species of *Staphylococcus* were mainly responsible for subclinical mastitis, but some agents, like *Streptococcus agalactiae* were found in both clinical and subclinical mastitis. The same authors also reported that also reported from Kenya that *Streptococcus agalactiae* and *Staphylococcus aureus* were found in 12.1 and 10.6% respectively, of camel milk samples in Kenya, whereas they were found in 9.6 and 16.9% respectively of milk samples in the present study. The high frequency of isolation of *Streptococcus agalactiae* and other major mastitis pathogens could be attributed to the lack of

supply and infrequent use of antimicrobials and the inaccessibility of the camel owners to veterinary services as compared to dairy cow owners in urban and peri urban areas. Although they were rapidly destroyed by inflammatory reactions, *E. coli* and other Coliforms quite commonly provoke a higher CMT score. Even though the proportion varies, mastitis pathogens isolated in the present study were also reported in other studies [10, 13]. The epidemiology and pathogenesis of mastitis pathogens remains unclear as camels have not been a frequent subject of research [28]. Hence, the treatment approaches and their results in traditionally managed camels are questionable [29].

The use of ant-suckling devices in Borena camels locally called '*Mara*' is practiced only during day time when young calves older than one year are herded together with their dams. The use of this device together with heavy tick infestation could predispose the udder to bacterial infestations, which persist as chronic infection. This could result in indurations and atrophy of injured quarters [23]. Other non-traumatic devices such as the ones used in Mauritania, a protecting harness made of rope named "Shmell" [30], could serve alternatively to reduce injury incidence. In addition to camels, nomads in Borena kept other animals such as cattle and goats which could be sources of some mastitis pathogens [23]. Moreover, the udder is a predilection site for tick infestation which causes udder skin and teat lesions, facilitating bacterial entry and leading to permanent tissue damage. This may gradually damage the teat and udder, favouring bacterial infection and subsequent results in mastitis and udder lesions. As a result, significantly higher proportions of camels with udder lesion and tick infestations were mastitis positive. It was reported that 72% of the udders were infested by tick and the incidence of mastitis was higher in heavy infested (30%) than in non infested (9%) udder [10]. Similarly, Husein *et al*. [12] have also observed an association of mastitis prevalence with tick infestations and udder lesions. Therefore tick control using acaricide treatment may reduce mastitis and udder health problems in camels. Abdurahman [28] has suggested the income of removing ticks even when animals are not lactating by gentle rotations and firm downward motions without damaging the udder skin. Other udder health problems of the studied camels included among others, lesions or wound and udder deformities. A total of 26 (7.5%) animals have lesion on udder and teat in the present study. It has been demonstrated that udder lesion due to tick, thorny bushes and cauterization of udder skin and poor udder hygiene are the major hindrance to overcome the udder health issues of camels [13, 28]. These conditions may be directly responsible to mastitis by causing injury and predisposing the udder to bacteria invasion. Additionally, injury and laceration wounds were found to be further infested by fly larvae leading to severe complications. Similarly, abnormal udder conformations were also common among the studied camels. Conformational problems including small nipple, large bulbous nipple and divergence from the vertical axis were reported in Camelidae [31]. These abnormalities may be the result of chronic mastitis or blockage of the teat canal or excessive udder lesions. Although there is dearth of information about the economic loss due to mastitis, it is not an obscured issue to imagine the potential losses associated with mastitis and blocked teat canals. The present study showed that 2.2 and 7.5% at quarter and animal levels respectively exhibited a blocked teat canal. Taking only clinical mastitis and blocked teats into account, 13.2% of camels had no sound teat for milk production. Additionally, camel mastitis reduces milk production, indeed, affecting food security and survival of the pastoral households where camels are the major source of food.

This study revealed that parity, lactation stage and age have significantly affected the mastitis (clinical and subclinical) prevalence. Globally, the frequency of mastitis has increased according to the age, parity number and stage of lactation probably because of an insufficient treatment efficacy leading to chronic mastitis and a possibility to carry bacteria over from first to the next parity. Poor body condition has also had a significant effect over good and medium body conditions. This could be associated with reduced defence status of the animals, increasing their susceptibility to udder infection by opportunistic organisms. Association of poor body conditions with increased mastitis prevalence has been already demonstrated in dairy cattle [32]. Several factors may contribute to poor body condition including malnutrition or parasitic infection and old age compounded with reproduction stress.

CONCLUSIONS

The present study showed that udder health problems are very common in the study area, mastitis was the major problem investigated in the study area in camel population. Moreover, the increase risk of acquiring mastitis with the various risk factors (age, parity, lactation stage and body condition score) was evident in this

study, which makes management factors to be an important in reducing mastitis infection. In light of this awareness should be developed within the herders to give more attention to udder health and also prevent mastitis. Further researches should be conducted in the future to monitor udder health and milk quality in traditional camel breeding.

Authors' Contribution: Tesfaheywet Zeryehun conceptualized and implemented the study design, involved in the acquisition of data and data analysis. Tesfaheywet Zeryehun, Godana Harro and Bedane Adane involved in data collection and laboratory analysis and also drafted the manuscript. All the three authors revised the manuscript, read and approved the final manuscript.

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