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Study on Prevalence and Risk Factors of Bovine Mastitis in Borana Pastoral and Agro-Pastoral Settings of Yabello District, Borana Zone, Southern Ethiopia

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Abstract: The study was conducted to determine prevalence of bovine mastitis, identify predominant bacteria responsible for mastitis infection and assess potential risk factors associated with the disease. A total of 460 lactating Boran breed cows from both pastoral and agro-pastoral set up of the district were included in the study. California Mastitis Test (CMT), clinical examination of udder and teats and bacteriological examination were employed during the study period. The overall prevalence of mastitis at a cow level was 59.1% (272/460), from which 21.1% (97/460) and 38.0% (175/460) were clinical and subclinical, respectively. The quarter level prevalence of the disease was also 38.7% (712/1840) from which 13.4% (246/1840) and 25.3% (466/1840) were clinical and subclinical form, respectively. Among the cause of bovine mastitis in the study area *Staphylococcus* species, *Streptococcus* species and *E. coli* were leading infectious causes with relative percentage of 29.2%, 22.5% and 11.4%, respectively. All the potential risk factors considered in this study namely, parity ($\chi^2 = 83.6$, $p = 0.00001$), age ($\chi^2 = 16.4$, $p = 0.0003$) and stage of lactation ($\chi^2 = 14.1$, $p = 0.0009$) showed very highly significant effects on prevalence of mastitis in the present study. Thus, high prevalence was observed in older cows >10 years and cows with parity >7 calves. In general, management practices and hygiene of dairy environment in all studied pastoral associations were very poor. Adequate sanitation of dairy environment, proper attention to health of mammary gland, regular screening tests and awareness of the people of the area about the disease should get emphases as control strategies and antimicrobial sensitivity tests for the isolated bacterial species were recommended for further study.

Key words: Boran Cattle · California Mastitis Test · Pastoral Production · Agro-Pastoral Production · Prevalence · Risk Factors

INTRODUCTION

Cows represent the largest proportion of cattle population of Ethiopia in which 55.5% of the total cattle heads for the private holdings are milking cows [1]. However, competed to other country in Africa, Ethiopia consumes less dairy cattle products. Per capita consumption of milk in Ethiopia is as low as 17 Kg per head while the average figure for Africa is 26 Kg per head. Given the considerable potential for smallholder income and employment generation from high value dairy products, development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country [2]. Milk which is a very nutritional food i.e. rich in carbohydrate, proteins, fats, vitamins and minerals, provides an important dietary source for the majority of rural as well as a considerable number of the urban and peri-urban population [3]. However, its reduced production, quality deterioration is a great concern in association with health risk to consumers due to the presence of zoonotic pathogens and antimicrobial drug residues.

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The quality of milk may be lowered by a number of factors such as adulteration, contamination during and after milking and the presence of udder infections. Pathogenic organisms in milk can be derived from the cow itself, the human hand and utensils or the environment [4]. Mastitis is one of the most complex diseases of dairy cows that generally involve interplay between management practices and infectious agents, having various causes and degrees of intensity [5]. It is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the world's dairy industry [6]. Transmission occurs mainly at milking time through contaminated milking machines, clothes and hands of milkers or machine operators [7]. In present day of Ethiopia, there is a national drive to alleviate the existing food deficit by devising different agricultural strategies including improvements of the productivity of livestock sector by controlling some of the major infectious diseases through regular monitoring scheme [8]. Mastitis, as a disease, has received little attention in Ethiopia, especially the subclinical form which is mainly caused by *Staphylococcus aureus* [9, 10]. Efforts have only been concentrated on the treatment of clinical cases. Owing to the heavy financial implications involved and the inevitable existence of latent infection, it is obviously an important factor that limits dairy production. The disease should be studied as it causes financial losses as a result of reduced milk yield and quality, discarded milk following antibiotic therapy, veterinary expense and culling mastitic cow [9]. In the same way, when modern dairy farming in the tropics was first adopted, mastitis was predicted to be an important disease in dairy cattle. Several studies conducted in different corners of the country indicated a prevalence range of 2.7-21.0% for clinical mastitis and 33.3-68.1% for subclinical mastitis cases in small and large scale dairy farms. In general mastitis is of great economic importance to all dairy producers and also in pastoral and agro-pastoral dairy production systems in Borana zone as reported by Bedane *et al.* [11] who has indicated in an assessment study that the impact of ticks on cattle milk production in pastoral and agro-pastoral settings of Borana zone had caused damages to teats, ending up in its blinding and mastitis disease and thus set recommendations of further research direction to investigate mastitis infection it causes and other contributing factors. Therefore, the present study was initiated with this background and consideration to determine the prevalence rate of mastitis in the study area, isolate bacterial species responsible for the infection and to determine predisposing risk factors for the disease.

MATERIALS AND METHODS

Study Area: The study was conducted from November 2010 to April 2011, in and around Yabello district, Borana Zone, Oromia Regional State, Ethiopia. The Borana Zone (Southern Rangeland), is considered as the finest grazing land in East Africa [12]. The zone is endowed with large population of livestock [13]. The Yabello district comprises about 23 Pastoral Associations (PAs) in which 48% (11 PAs) and 52% (12 PAs) of the peoples dwelling in and around the district practice pastoral and agro-pastoral activities, respectively [14].

Study Animals and Husbandry Practices: The study animals were the local zebu (lactating Boran breed dairy cows), which are managed under traditional and extensive husbandry systems in the selected pastoral associations (PAs') of the study area. The Boran breed is relatively medium size with big udder and long teats. Animals are maintained usually in simple enclosure with no distance from each other. Enclosure cleaning and dung removal is not made regularly on daily basis. Although milking is done by hand, pre-milking as well as post-milking hygienic procedures, such as udder washing and drying, are less frequently practiced or nil at all.

Sample Size and Sampling Strategy: The study involved a multistage random sampling technique to select study pastoral associations, villages, households and animals, randomly 6 pastoral associations of which 50% (3 PAs') from pastoral and the remaining 50% (3 PAs') from agro-pastoral were selected. Then from each selected PA's, 3 villages were selected randomly and finally appropriate herds were selected from each village followed by sampling lactating cows from each randomly selected herd. Accordingly, a total sample size of 460 lactating cows from smallholder dairy herds were determined by assuming the expected prevalence of 50%, with the confidence interval of 95%, while the desired precision set was 5% for the presence of clinical mastitis, subclinical mastitis and associated risk factors based on the formula described by Thrusfield [15].

Collection of Milk Samples: Milk samples were collected according to the standard procedure [8]. Approximately 10 ml of milk was then collected aseptically from lactating cows into sterile test tubes after discarding the first three milking streams. Samples from each quarter was transported in ice box (4°C) to Yabello Regional Veterinary

Laboratory, where the samples were immediately processed or stored at 4°C until processed or cultured on standard bacteriological media [8].

Study Design

Cross-Sectional Study: The California Mastitis Test (CMT) [5] was carried out in the field as a screening test for subclinical mastitis and for selection of the samples for the bacterial culturing from the cows under study.

Observational Study: The lactating cows were clinically observed for the manifestation of general clinical signs related to udder and presence of any gross lesions like fibrosis, inflammatory swellings, pain, visible injury, atrophy of the tissue and swelling of supramammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, pain upon palpation and blindness. Physical appearance of milk including color, odor, consistency, specific gravity, viscosity and appearance of milk secretion from each mammary quarter with the presence of clots, flakes, blood and watery secretions were also used for screening of presence of clinical mastitis [5].

Bacteriological Examination of Milk Samples: Milk samples were examined following standard procedures where about one standard loop full (0.01ml) of each milk sample was streaked on MacConkey agar and tryptone blood agar base enriched with 7% sheep blood. Plates were incubated aerobically at 37^0C for up to 48 hrs and checked for any bacterial growth. Positive bacterial cultures were transferred into nutrient agar and further identification of the bacterial species was done on the basis of Grams reaction, colony morphology and biochemical tests and plating on selective media [16].

Risk Factors: A semi-structured questionnaire format was developed and pre-tested with the primary objective of elucidating the multifactorial background of mastitis and assessing the awareness of the people in the study area about the disease. Data collected include age, parity, lactation stages and management of dairy cows (housing, milking practice, history of mastitis in the herd). Udder and milk abnormalities (injuries, blindness, swelling, milk clots and abnormal secretion) were also recorded. Age of the study cows was determined by the information from the owner and dentition characteristics [17] and categorized as young adults (≥3 to 5 years), adults (≥6 to 9 years) and old (>9 years). Parity was also categorized as few (with 1-3 calves), moderate (4-7 calves) and many (>7 calves). Lactation stage of the cow was categorized as early (1-4 months), medium (5-8 months) and late (>8 months up to the onset of dry period).

Data Management and Statistical Analysis: The data collected during the study periods were entered into MS-Excel spread sheet and analyzed using SPSS version 15 software. The statistical analysis used included comparison of proportions and Chi-square test, which was applied to test if statistically significant association existed between predisposing risk factors such as age, lactation stage and parity of cows with mastitis positivity. For all the analysis performed $p<0.05$ was taken as statistically significant [18].

RESULTS

Prevalence of Mastitis: Out of a total of 460 lactating cows examined 59.1% were positive for mastitis, of which 21.1% (97/460) and 38.0% (175/460) were clinical and subclinical mastitis, respectively. The quarter level prevalence of mastitis was 38.7% (712/1840), where clinical and subclinical mastitis cases account for 13.4% (246/1840) and 25.3% (466/1840) of the cases, respectively. Table 1 summarizes the prevalence of mastitis in the 6 PAs' at cow and quarters levels. From the total positive cows, 152 and 120 of them were found positive in pastoral and agro-pastoral settings, respectively, whereas, 366 and 346 of examined quarters were found positive in pastoral and agro-pastoral settings of the district, respectively.

Bacteriological Examination: The bacteriological examination results revealed that milk samples from 577 (81.0%) quarters were found positive upon culturing on bacteriological media out of a total of 712 CMT and clinical examination positive quarters. The relative occurrence of bacterial species is described in (Table 2), where *Staphylococcus* species accounted for the highest percent (29.2%) followed by *Streptococcus* species which accounted for 22.5%.

Potential Risk Factors: The statistically analyzed data summarized in (Table 3), revealed the presence of significant effects of potential risk factors on the prevalence of both subclinical and clinical mastitis in

Table 1: Overall prevalence of mastitis in all six selected pastoral associations

		Cow level		Quarter level	
	Area settings	Positive	Prevalence (%)	Positive	Prevalence (%)
Pastoral	Surupha	63	13.7	152	8.3
	Arweyu	53	11.5	127	6.9
	Haro-bake	36	7.8	87	4.7
Agro-pastoral	Elwayou	56	12.2	143	7.8
	Cholkassa	41	8.9	128	6.9
	Dida-Yabello	23	5.0	75	4.1
Total	6 PAs	272	59.1%	712	38.7

Table 2: Relative frequency of bacterial species from clinical and subclinical mastitis cases by bacteriological examination

Sample cultured	Bacterial isolates	Numbers of bacterial isolates	Proportion (%)
On blood agar	Staphylococcus species	208	29.2
	Streptococcus species	160	22.5
	Other Gram positive cocci and Rods	3	10.3
	E. coli	81	11.4
	Other Gram negative rods	125	17.6
Total positive		577	90.0
Total negative		64	10.0
Overall total		641	100.0%

Table 3: Association of potential risk factors with prevalence of mastitis at cow level

Risk factors	Groups	Total	CMT positive No. (%)	χ^2 = Cal df = 2	P_Value
Age	3-6	116	74 (63.8%)	16.4	0.0003
	7-10	154	86 (55.8%)		
	>10	190	145 (76.3%)		
Parity	<3	189	77 (40.7%)	83.6	0.00001
	4-7	115	79 (68.7%)		
	>7	156	137 (87.8%)		
Lactation stage	1-4	118	57 (48.3%)	14.1	0.0009
	5-8	197	137 (69.5%)		
	>8	145	89 (61.4%)		

the study area. Accordingly, all the three risk factors considered and assessed in the study namely, parity numbers (χ^2 = 83.6, p = 0.00001), age (χ^2= 16.4, p = 0.0003) and stage of lactation (χ^2 = 14.1, p = 0.0009) indicated to have significant effects on prevalence of mastitis.

Clinical and subclinical mastitis encountered at different stages of lactation were significant ($p<0.05$). This information is supplemented with higher number of cases resulted in those at 5-8 months of lactation and greater than 8 months to until the onset of dry period. The percentage of mastitis cases were 48.3% (57/118), 69.5% (137/197) and 61.4% (89/145) for few, moderate and many parity groups, respectively. Among the three considered risk factors there was highly significant differences for parity and lactation stage, ($p<0.01$) and significant difference for age ($p<0.05$) in the prevalence of mastitis.

Questionnaire Survey Results: All the households visited during the study period practiced hand milking in which mastitis was reportedly detected only by milk change and udder inflammation. Meanwhile, 86.7% (26/30) of the households reported the presence of mastitis in their herd. In 83.3% (25/30) of the households mastitic cows were milked without any order where as 16.7% (5/30) of cows were left without milking at all after infected. Within the visited pastoral associations clinical mastitis was recorded more than the subclinical mastitis in the pastoral setting than the agro-pastoral setting.

DISCUSSION

In the present study the overall prevalence of mastitis in local Boran breed cow was 59.1%, which can be of significant value regarding the economic and public

health importance of the disease to the study area. The overall mastitis prevalence reported in the present study is in close agreement with previous findings of other authors in different regions of Ethiopia like: 61.1% by Tolla [19] in south Wello, 63.0% by Geressu [20] in Addis Ababa. However, the prevalence reported in this study is lower than the previous report of 68.1% by Zerihun [21] in Addis Ababa and 85.6% by Nesru [22] in Dire-Dawa. However, overall mastitis prevalence reported in the present study is higher than the previous findings of other authors in different regions of Ethiopia like 34.9% by Biffa *et al.* [23] in Southern Ethiopia, 36.9% by Darsema [24] in Dire-Dawa Eastern Ethiopia, 38.6% by Fekadu in the Chaffa valley [25] 39.5% by Geressu [26] in Debre-Zeit in central Ethiopia, 44.6% by Mengistu [26] in Bahir-Dar, 45.9% by Shimelis [27] in Soddo, 46.7% by Abera *et al.* [28] in Adama, 49.7% by Enquebahir *et al.* [29] in Tigray, 52.8% by Hundera *et al.* [9] around Sebeta, 53.0% by Takele [30] in Arsi, 53.5% by Tolossa [31] in Kallu province and Abdelrahim *et al.* [32], who found a prevalence of 45.8% in Sudan and Radostits *et al.* [5] who described the prevalence of mastitis to be around 50% in cows in most countries irrespective of the causative agent. The differences in prevalence reports of mastitis in the present study and other reports could be attributable to difference in breeds of targeted cows, farm management practices, level of production and differences in study methods and materials employed by the investigators. In this study, the prevalence of subclinical mastitis is higher (38.0%) than clinical mastitis (21.1%) which is supported by reports of several investigators 21.0% and 44.0% by Lemma [33], 6.3% and 47.2% by Tolossa [31] and 12.5% and 41.2% by Takele [30] for clinical and subclinical mastitis, respectively. The prevalence of subclinical mastitis in this study is in close agreement with, 36.7%, 36.7%, 39.5%, 39.6%, 38.2%, prevalence reported by many investigators [9, 20, 26, 28, 35], respectively. The prevalence report of clinical mastitis is also closely related with reports of several other investigators; 21.1% by Tarekegn [21] and 21.0% by Lemma [33] in Arsi region and 20.0% prevalence reported by Bagadi [37] in Sudan. The reports of Biffa *et al.* [23] and Almaw *et al.* [34] on the prevalence of subclinical mastitis (23.0% and 25.2%, respectively) are lower than the results of the present study. In general, subclinical mastitis has been reported to be higher than clinical mastitis owing to the defense mechanism of the udder, which reduces the severity of the disease [38]. In most developing countries including Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases [39]. The quarter level prevalence of mastitis in both agro-ecological settings is in close agreement with the works of other authors [2, 28 and 29]. However, it is higher than previous reports [22, 26, 27, 39 and 40]. It has been suggested that variation in the quarter prevalence of mastitis in different reports is directly related with the variation in the overall prevalence of the disease in the areas.

In the present study, Gram positive bacteria comprises 62.2% where as 29.2%, 22.5%, 10.3% of the isolates were found to be *Staphylococcus* species, *Streptococcus* species and other Gram positive cocci and rods, respectively and these were the major pathogens isolated from both clinical and subclinical mastitis. *Staphylococcus* and *Streptococcus* species are the two major Gram positive cocci commonly associated with bovine mastitis in which *Staphylococcus* species constitute major percentage (29.2%). The high prevalence of *Staphylococcus* species in the present study is in agreement with findings of several other investigators [2, 19, 20, 25, 29, 33]. Staphylococci are the most important and prevalent mastitis causing organisms globally, including Ethiopia [2, 19, 29]. This organism causes contagious mastitis and primarily resides in the mammary gland of cows.

The isolation of streptococcal organisms from mastitis cases during this survey (22.5%) was lower than the incidence reported for the same species by many investigators [32, 41, 42] (81.0%), (53.6%), (27.0%) and higher than report of Matios *et al.* [2] (14.2%), in dairy cows. The most commonly used antibiotic for the treatment of mastitis cases in the study area is penicillin in combination with streptomycin and this may be the probable reason for lower isolation rate of this organism and is also supported by other evidences as mastitis caused by *Streptococcus* species is susceptible to eradication via use of penicillin [5, 16]. In the present study, the high incidence of coliform mastitis could also probably be due to poor hygienic conditions, as these organisms like *E. coli* originates from the cow's environment and infect the udder via the teat canal [43]. Formerly, contagious mastitis accounted for most outbreaks of the disease both in the developed and developing world but, following the implementation of mastitis control program during the last three decades, the incidence of contagious mastitis due to *Staphylococcus aureus* and *Streptococcus agalactiae* has been declined in the developed world but, still remained as major cause of mastitis in the developing world. This fact is also supported by the questionnaire survey of the present

study in the different PAs'. In those herds, where there were poor hygienic and milking practices, the incidence of *Staphylococcus* and *Streptococcus* species was higher that other organisms as these organisms exist in the mammary gland of the cow and the major root of transmission for these organisms occur during milking through milkers' hands, teat cup liners and udder clothes [44]. The results of this study are in line with this fact as all the herds visited use hand milking and washing hands, udder and teats before milking is not practiced. In contrast, the incidence of coliform organisms like *E. coli* was higher in clinical mastitis and in herds with poor housing (drainage, barn cleaning and often muddy bedding) conditions in the present study. Contamination of teat ends is a major predisposing risk factor in the development of environmental mastitis, due to the fact that environmental pathogens can survive and multiply in organic bedding materials and housing conditions that can influence teat contamination rates [44]. The observed higher prevalence of mastitis with increasing age is in accordance with the works of several investigators [28, 31] who found that, the risk of clinical and subclinical mastitis increase significantly with the advancing age of the cow. Prevalence of mastitis was also significantly associated with parity (x^2 = 9.18, p = 0.01). There was higher occurrence of mastitis in cows with >7 calves than those cows with 1-3 calves. This finding is in agreement with findings of Takele [29]. In addition to the above mentioned factors, the lactation stage of cows was also found to have highly significant effect on the prevalence of mastitis (x^2= 10.17, p = 0.006 [30]. In the present study, the prevalence of the bovine mastitis was very high in both pastoral and agro-pastoral associations of the study settings, which is beyond the economically tolerable limit. Major bacterial pathogens such as *Staphylococcus* species, *Streptococcus* species and *E. coli* are found the predominant pathogens responsible for infections of the udder. There is strong association of potential risk factors (age and parity) with prevalence of mastitis in the study area. Inadequate hygienic condition of the dairy environment, poor animal health services, absence of hand and udder washing before milking and lack of practicing of dry cow therapy are important predisposing risk factors of mastitis in the study area. Better management practices in milking and housing should be properly organized and delivered to agro-pastoralists and pastoralists in the zone and practical mastitis controlling strategies should be initiated and promoted. A well designated further investigation should be devised to assess the antimicrobial susceptibility of predominant causative bacterial pathogens.

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