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Keywords: *CMT, bacterial culture, mastitis, prevalence, bovine, borana, north showa.*

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Prevalence, Isolation of Bacteria and Risk Factors of Mastitis of Dairy Cattle in Selected Zones of Oromia Regional States, Ethiopia

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Abstract- A cross sectional study was conducted on a total of 471 cross and pure borana breed dairy cattle to determine prevalence of clinical and subclinical mastitis using CMT in selected districts of North Showa and Borana zones of pastoral area from April 2012 to February 2014. The overall mastitis prevalence was 237(50.3%). The cow level prevalence was 9.5% clinical and 40.7% were subclinical cases. Of 1884 quarters examined 10(0.5%) quarters were blind teats and quarters 550(50.2%) were showed mastitis. High score CMT positive milk sample were investigated using standard microbiological techniques. Identification of bacterial isolates revealed that 10 types of bacterial isolates were identified. The isolated bacteria were *Staphylococcus aureus* and CNS 20 (37.7%), *Diplococcus* spp 2 (3.8%), *Corynebacterium pseudotuberclosis* 3(5.8%), *Corynebacterium bovis* 1(1.7%), *Micrococcus* spp 1(1.9%), *Pseudomonas* spp 1(1.9%), *Bacillus* spp 1(1.9%), *E. coli* 3(5.7%) *Proteus* spp 1(1.9%). Different risk factors like parity number, farming hygiene, animal origin and husbandry type were considered. Hygienic conditions and husbandry type were the most important potential risk for mastitis. Statistically, cattle from North Showa were highly infected than those from Borana zone ($p < 0.05$). Farmers and herd managers should give great attention for hygiene condition and husbandry type and further investigation should be conducted especially in the pastoral area.

Keywords: CMT, bacterial culture, mastitis, prevalence, bovine, borana, north showa.

I. Introduction

Ethiopia holds large potential for dairy development due to its large livestock population and the favorable climate for improved and high yielding breeds. Ethiopia has the largest livestock population among African countries (CSA, 2008).However, compared to other countries in Africa, Ethiopians consume less dairy products. Moreover, the quality and quantity of milk in the country deteriorates because of various causes. Given the considerable potential for generation of income and employment, the development of small holder dairy sector in Ethiopia, has a promising future and can contributes significantly to poverty alleviation improved nutrition in the country. According to Ahmed *et al.* (2007), milk production during the 1990s expanded at an annual rate of 3.0% compared to 1.63- 1.66% during the preceding three decades, with the expected growth in income, increased urbanization and improved policy environment (Kelay, 2002).The central highlands, mainly Selalle, are the major dairying areas, and as a result they are the main sources of milk for Addis Ababa, Ethiopia's capital and main urban population center, where 8% of its inhabitants live. Furthermore, farmers in Selalle are conscious of the milk market and produce milk for commercial sale, unlike the majority of Ethiopian farmers,who produce milk for home use. In Selalle farmers keep high-yield cross bred (zebu * Holstein) and Holstein dairy cattle mainly for milk production alongside native zebu breeds (Ameni *et al.,* 2007). Study by Kasim *et al.* (2012) indicated pure Borana breed are more productive than other local breeds in Ethiopia. However, milk production often does not satisfy the country's requirements due to a multitude of factors among them udder infection is the one (Erskine, 2001).

Mastitis is an inflammation of mammary gland, primary resulting from invasion of the mammary gland by pathogenic microorganisms through the teat canal resulting in physical, chemical, pathological and bacteriological changes in glandular tissues and milk. Many risk factors have been identified for clinical and subclinical mastitis in dairy animals such as breed, increased milk production, hygienic conditions, milking practices, age, parity, stage of lactation (Fox *et al*., 1995; Barnouin, and Chassagne, 2001).

The most common pathogen comprises contagious bacteria mainly *Staphylococcus aureus* and *Streptococcus agalactae* and environmental bacteria mainly coli forms and some species of streptococci that are commonly present in the environment (Radostitis *et al.,* 2007).Besides, mastitis may render milk unsuitable for human consumption or provide a mechanism for the spread of diseases like Tuberculosis, Streptococcal intoxication, Colibacillosis, streptococcal sore throat and Brucellosis to human

Bovine mastitis was reported as one of the most prevalent dairy health problems in Ethiopia including north Showa and Borana zones (Argaw and Tolosa,

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2008). Yet, the information on the prevalence of sub clinical mastitis in the areas is lacking and what available is fragments of information from cases of clinical mastitis that has been presented to veterinary clinic for the treatment. Therefore, the objectives of this investigation were: to determine the prevalence and major risk factors associated with clinical and sub clinical mastitis at herd, cow and quarter level in small holder and pastoral area dairy cattle.

II. Materials and Methods

a) Study area

This study was carried out in North Shoa Zone of the Oromia Regional state in central Ethiopia and Borana pastoral and agro pastoral zone from April 2012 to February 2014. NorthShoa Zone is located in central Ethiopia 126 km north west of Addis Ababa in Oromia Regional state. It covers 1,174,500 hectare of land from which 40% is crop land, 25% is grazing land, 13% is forest and bush area, 7% is construction area and 15% is unproductive land. It's minimum and maximum temperatures vary from 11.5-29°C and 17.5-35°C, respectively. It gets bimodal rainfall that ranges from 651-1115mmi.e. from February-May (short rainy season) and from June- October (long rainy season) (North Shoa Agricultural Department, 2013).In particular Girarjarso, Debrelibanos and Wachale districts of North Showa were our study area.42% of the area is highland that is suitable for crop cultivation and livestock husbandry and the herd structure is characterized by a higher number of cows. Sample collection areas will be in 10 km radius of the milk collection units along the main road in both sides.

The study also conducted in Miyo District of Borana zone. The district is located at 717 Kms south of Addis Ababa. This district is characterized by pastoral and agro-pastoral production systems. The livestock species reared within the district are cattle (61,023), goat (72,224), sheep (14,567), camel (15,672), equines (12,613) and poultry (11,236). The rainfall pattern is bimodal in nature with average annual rainfall around 700mm. The average annual temperature is 22 °C. The altitude of the district ranges from 1300-1520 m.a.s.l (CSA, 2008).

b) Study Population

The study were conducted on a total of 144 heads of cross (Zebu and HF) breeds(of lactating cows kept under small holder dairy herds kept under extensive, semi- intensive and intensive husbandry practice in Salale of North Showa, and on327 pure Borana breed characterized by both meat and milk production merits.

c) Study design

A cross sectional study was conducted on small scale holder dairy farms in North Showa, and pastoral and agro pastoral area in Borana of Southern Ethiopia. Data on each cow was collected in a format designed for this purpose. The data sheet mainly focused to address associated risk factors with the occurrence of bovine mastitis. Risk factors considered were cow history, housing system, milking practice, hygiene, parity number, stage of lactation, husbandry type and other management practices in the study area.

d) Sample Size determination

The sample size was calculated according to the formula given by Thrusfield (2007). In Salale of North Showa it is calculated by taking (89.54%) prevalence from previous report by Argaw and Tolosa (2008).In Miyo of Borana considering the previous prevalence 59.3% in and around Yabello district (Kasim *et al.,* 2012), a total of 374 lactating dairy cows were proposed to be sampled but, attributable to the2010/11 drought shock of the zone only 327 heads of cows were included.

e) Sampling Procedure

To include cows from small scale holder dairy farms in selected districts of North Showarandom sampling method was employed to select the individual dairy cow. Selection was done by judgment mainly following accessibility to the main road in 10 km radius which is supplying their milk to eight primary farmer milk cooperatives in the three study districts. In Miyo of Borana purposive sampling was used to test lactating dairy cows available within the district due to the fact that the number of cows within the district are limited as a result 2010/11 drought shock in the zone.

f) Study Methodology

i. *Clinical Inspection of the Udder*

The clinical inspection of the udder was done in the following way. The udder was first examined visually and then by palpation to detect fibrosis, inflammatory swellings, visible injury, atrophy of the tissue, and swelling of supra mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Information relating to the previous health history of the mammary quarters and causes of blindness was obtained from interviews with owners. Viscosity and appearance of milk secretion from each quarter were examined for the presence of clots, flakes, blood, and watery secretions (Quinn *et al.,* 2002).

g) Laboratory test

i. *California mastitis test (CMT)*

The California mastitis reagent was used to screen cows with subclinical mastitis when milk sample is collected according to the procedures of National Mastitis Council (NMC, 1999). Milk sampled according to National Mastitis Council, (NMC, 1999) was subjected to California mastitis reagent to screen subclinical

mastitis described by (Quinn *et al.,* 2002).This test is based on increased number of leucocytes and increased alkalinity in milk due to mastitis (as an indirect measurement of leucocytes). 0.5ml of milk from each quarter is taken in plastic peddle cups and added to equal quantity of CMT reagent solution and mix well by circular movement of peddles mixed on a horizontal plane. The result of the test indicated on the basis of gel formation .Depending on clinical inspection and CMT results, cases were categorized as either positive or negative. Positive cases were further categorized as clinical and sub-clinical mastitis. The interpretation (grades) of the CMT was evocated and the results was graded as 0 for special chemical concentrated commercially prepared negative and trace 1, 2 and 3, for positive (Quinn *et al.,* 2002).

h) Microbial investigation of mastitis

i. *Milk sample collection*

The milk sample was taken from cows not treated previously with either intra mammary or systematic antimicrobials agents. For good collection of sample the teat was wiped thoroughly with 70% ethyl alcohol. Procedures for collecting milk sample were according to (NMC, 1999; Quinn *et al*., 2002). Strict aseptic procedures were used when collecting milk samples in order to prevent contamination with the microorganisms present on the skin of cow's flanks, udder and teats, on the hands of the sampler, and in the barn environment. Teats towards sample collection were taken first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was as near horizontal as possible and by turning the teat to a near horizontal position. The milk sample was held in an ice box and transported immediately to the laboratory for culturing.

i) Bacteriological isolation and biochemical characterization

Culturing of milk sample was performed according to microbiological producers of (Quinn *et al*., 2002 and Radostits *et al*., 2007) for the diagnosis of bovine mastitis. Briefly, a loopful of milk sample collected from each infected quarter was inoculated separately on to MacConkey agar and blood agar base enriched with 5% defibrinated sheep blood. The inoculated plates were then incubated aerobically at 37^0C for 24 to 48 hours. Identification of the bacteria on primary culture was made on the basis of colony morphology, hemolytic characteristics, Gram stain reaction including shape and arrangements of the bacteria, catalase and O-F tests. Staphylococci were identified based on Catalase test, growth characteristics on Mannitol salt agar and purple agar and tube coagulase test. Gram negative isolates grown on MacConkey agar were identified based on growth characteristics on MacConkey agar, Oxidase reaction, Catalase test, triple sugar iron (TSI) agar ,the "IMViC" (indole ,methyl red, Voges-Proskaur, and citrate) test (Quinn *et al.,* 2002).

j) Data Management

The collected data about history, clinical inspection, CMT, and result of bacterial culture were evaluated using SPSS soft- ware (SPSS 20.0 version) with Chi-square ($\chi 2$) and $p < 0.05$ was considered statistically significant.

III. Results

Of a total 471 examined lactating cows the overall prevalence of mastitis in the study areas was 237 (50.3%).The result showed that the prevalence of clinical and subclinical mastitis were 9.5 % and 40.7%, respectively. The result also indicated prevalence of bovine mastitis in north Showa zone is higher as compared to the Borana zone (Table.1).

Table 1 : Prevalence of Bovine Mastitis in Borana and North Showa zone

Variable	Levels	No. examined	Prevalence (%)			P value
			Clinical	Sub clinical	Subtotal	
Zone	Borana	327	40(12.2)	70(21.4)	110(33.6)	0.00
	North Showa	144	5(3.5)	122(84.7)	127(88.2)	
Districts/PA	Mio	68	10(14.7)	16(23.5)	26 (38.2)	0.00
	Baha	80	6(7.5)	20(25.0)	26(32.5)	
	Boku-luboma	83	12(14.4)	14(16.9)	26(31.3)	
	Hiddi	35	3(8.6)	5(14.3)	8(22.9)	
	Dhokisu	61	9(14.7)	15(24.6)	24(39.3)	
	G/ Jarso	54	2(3.7)	50(92.60	52(96.3)	
	D/Libanos	49	2(4.1)	45(91.8)	47(95.9)	
	Wuchale	41	1(2.4)	27(65.9)	28(68.3)	
	Total	**471**	**45(9.5)**	**192(40.7)**	**237(50.3)**	

Out of 1884 quarters examined 10(0.5%) quarters were blind, leaving 1884 functional quarters. The prevalence of mastitis on quarter bases was 550 (29.2%). The result showed that higher infection rate in hindquarters as compared to the front quarters (Table 2).

Table 2 : The Prevalence of Mastitis at quarter level

Quarter	No. examined	No. positive (%)			No. blind
		clinical	subclinical	Sub total	
Right front	471	24(17.8)	111(82.2)	135(28.9)	4(0.8)
		X^2=199.9, P=0.00			
Right back	471	23(16.3)	118(83.7)	141(30.1)	2(0.4)
		X^2=202.0, P=0.00			
Left front	471	23(17.2)	111(82.8)	134(28.6)	3(0.6)
		X^2=192.3, P=0.00			
Left back	471	21(15)	119(85)	140(29.8)	1(0.2)
		X^2=203.3, P=0.00			
Total	1884	91(16.5)	459(83.5)	550(29.2)	10(0.5)

The result showed that the effect of lactation stage and parity number were statistically insignificant (P>0.05) on the prevalence of bovine mastitis. However, husbandry type and hygienic condition were statistically have significant differences on the infection prevalence (P=0.00) (Table. 3).

Table 3 : prevalence of bovine mastitis based on lactation stage,husbandry practice, hygienic condition and parity number

Risk factors		Result				P value
		No. examined	No. positive (%)			
			clinical	subclinical	Sub total	
Lactation Stage	Early	141	17(21.5)	62(78.5)	79(56.0)	
	Mid	111	7(14.6)	41(85.4)	48(43.2)	0.13
	Late	219	21(19.1)	89(80.9)	110(50.2)	
Husbandry	Extensive	17	0(0)	13(100)	13(76.5)	
	Semi Intensive	84	3(3.9)	74(96.1)	77(91.6)	
	Intensive	43	2(5.4)	35(94.6)	37(86.0)	0.00
	Pastoral	232	28(35.4)	51(64.6)	79(34.0)	
	Agropastoral	95	12(38.7)	19(61.3)	31(32.6)	
Hygiene	Good	74	2(3)	66(97)	68(91.9)	
	Medium	52	1(3.2)	30(96.8)	31(59.6)	0.00
	Poor	345	42(30.4)	96(69.6)	138(40.0)	
Parity number	1-3	338	38(21.7)	137(78.3)	175(51.8)	
	4-6	109	5(10.40	43(89.6)	48(44.0)	0.26
	>6	24	2(14.3)	12(85.7)	14(58.3)	
	Total	471	45(19)	192(81.0)	237(50.3)	

From the CMT positive samples, 63 milk samples were cultured and 53 bacteria were cultured on blood, nutrient agar for gram positive bacteria and Macconkey agar for gram negative aerobically. *S. aureus* and CNS (coagulase negative staphylococci), were the most isolated followed by *C. pseudotuberclosis* and *E. coli* and the other such as *Diplodocus* Spp., *C. bovis, Micrococcus* Spp., *Bacillus* Spp., and *Pseudomonas* Spp. were rarely isolated as causative agents of mastitis. (Table 4).

Table 4 : Prevalence of isolated bacteria in the study areas in (2012-2014).

Bacteria	Frequency	Proportion%
S. aureus	20	37.7
CNS	20	37.7
Diplococcus species	2	3.8
C.pseudotuberclosis	3	5.7
C. bovis	1	1.9
Micrococcus species	1	1.9
Pseudomonas	1	1.9
Bacillus species	1	1.9
E .coli	3	5.7
Proteus species	1	1.9

IV. Discussion

Mastitis prevalence at cow level was found 50.3% which is highly disagreed with the report of Kifle and Tadele (2000) who reported 89.5% from North showa. This finding was also lower than the reports of Tariku *et al* (2011), Mekibib *et al* (2010) and Matios *et al* (2008) which were reported 75.2%, 71.3% and 65.5% respectively. However, it is slightly comparable with the finding of Birru (1989) who reported 43.5% from of Ethiopia. Mastitis is a multi –factorial disease and this difference may be due to herd size, agro- ecological and different managemental systems. In addition the present study shows the prevalence of mastitis is statistically higher ($p<0.05$) in north Showa than in Borana zone. As compared to the other districts/PAs of the study areaprevalenceof mastitis is highly important in w/jarso of the North showa and this may due to poor sanitation of the barn floor and use of organic bedding. Generally, the overall prevalence of mastitis reported from North Showa in the present study was higher than most of reports in the country and this could be due to major farmer depending on cross breed dairy cattle, absence of balanced diet feeding and lack of awareness about the disease.

From 1884 examined quarters, 550(50.2%) were CMT positive and 10(0.5%) quarters were blind. This finding is lower than that obtained by Kifle and Tadele (2000) and Birehanu (2008) as they reported 63.1% and 52.4% respectively at quarter level. Mekibib *et al.* (2010) reported that the prevalence of blind teat was 14% which is far higher than the present finding.

The prevalence of clinical mastitis at cow level was 9.5% and this is comparable with the reports of Molalegne *et al* (2010), Bedada and Hiko (2011) and Demelash *et al* (2005) who reported 10.3%, 10% and 11.9% respectively in different parts of Ethiopia. The current finding is little higher than the reports of Husien *et al* (1999) and Bishi (1998) who reported 5.7% and 5.3% respectively in different parts of the country. This may be due to concurrent disease involvement, interaction of several risk factors relating with animal and virulence of causative organism. In our study the rate of sub clinical mastitis is higher in pastoral production system than agro-pastoral (Table 2) attributable to higher number of animals in this system than agro-pastoral.

In this study, the prevalence of subclinical mastitis was 40.7% and this is lower than that reported by Argaw and Tolosa (2008) 89.5%. In addition, it is nearly similar to that founded by The finding is Bishi (1998) and Ahmed *et al*. (2007) However, our finding is higher than Mekibib *et al* (2010) who reported 25.2%. The high prevalence of subclinical mastitis may be due to improper milking hygiene, poor housing system, lack of post milking teat dipping.

Lactation stage showed statistically insignificant effect on the occurrence of mastitis $P>0.05$) which contradicts with the report of Birru (1989).Parity number has also no difference for occurrences of bovine mastitis.

In our study husbandry and hygiene showed statistically significant effect on the prevalence of mastitis ($p<0.05$). This could be due to different farming system, managemental practice and may be attributed to occurance of contagious mastitis and inability of control and physiology effect.

From the isolated bacteria the most dominant in the study area were CNS and *staphylococcus aureus* (37% for each) and the predominant causes of clinical and sub clinical mastitis in the area. This finding is little different from the finding of Molalegn *et al* (2010) who reported, (51.9%) of CNS. This study is compatible with the study of Mekibib *et al* (2010) and Abdella (1996) who reported (47.1%) and (31%) of *S. aures* respectively. In addition Tariku *et al.* (2011) reported 39.44% of *staphylococcus* species responsible for mastitis. However, the current finding is much lower than Workineh *et al.* (2002) who reported 70.5% of *Staphylococcus* species. The difference might be resulted from lack of effective udder washing and drying, inter cow hand washing and disaffection in the route of the area.

The other isolated bacteria were *E. coli* and *C. pseudotuberclosis (*5.56 % for each) and *Diplococcus* species 3.8%. This finding is lower than Hunder *et al.* (2005) who reported 14.2% of *C. Pseudotuberclosis* around Sebeta. Rarely isolated bacteria were *C. bovis, Baccilus spp, Micrococcus spp, Proteus spp* and *Pseudomonas spps* with equal ration of 1.9% for each. *B. cereus* and *B. subtilis* are saprophytic *Bacillus species* and the only mastitis causing pathogens (Radostits *et al.,* 2007).This result is in agreement with Tariku *et al* (2011) who reported (3.3%) *Micrococcus spp* and (2%) *C. bovis.*

In the current study the hind quarter have high mastitic prevalence and this may due to the hindquarter is more prone surface when the animal lay down touches hind leg in contact with contagious bacteria and to greater production capacity of hind quarter (Radostits*et al.,* 1994), likelihood of fecal accumulation, environmental contamination and difficulty of cleaning.

V. Conclusion and Recommendation

The overall prevalence of cattle mastitis is high as compared to most of previous reports in different parts of Ethiopia. Hygienic conditions and husbandry type were the most important potential risk for mastitis. The major isolated bacteria in this study were CNS and *S. aureus.* Farmers and herd manager are only concerned with clinical from of mastitis and often are unawareness of the status of subclinical infection in the

herd. The farmers have no enough understanding about effect of sanitation on the occurrence of the disease. Relaying on the above conclusion the following recommendation is for warded:

- Farmers and herd managers should give great attention for hygiene condition and husbandry type
- Periodic monitoring of infection status of the udder should be undertaken and positive animals should be treated using appropriate drug
- Careful milking practice and hygienic condition should be applied and the use of dry towel or sponge at least for each cow should be practiced
- Contaminated washing water and inappropriate bedding material that predispose to animals to mastitis should be avoided.
- Further investigation should be conducted on the area

Conflicts of interest

The authors have none to declare

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