Study On Prevalence Of Bovine Mastitis And Its Associated Risk Factors With Isolation And Identification Of *Staphylococcus Aures* And *Escherichia Coli* In And Aroun Asosa Town

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Abstract: A cross-sectional study was conducted in and around Asosa town from November 2018 to May 2019 to estimate the overall prevalence of bovine mastitis and its associated risk factors in cross breed and local zebu breeds of dairy cows, and to isolate *Staphylococcus aures* and *Escherichia coli* from mastitis cases. The physical examination of the udder, California Mastitis test and bacteriological examination were used in this study. Among the total 367 dairy cows examined for bovine mastitis, 127 were found positive with the overall prevalence of 34.6% in dairy farms found in and around Asosa town. Out of these, 7.9% (29/367) and 26.7% (98/367) were clinical mastitis and subclinical mastitis cases, respectively. The quarter level prevalence of mastitis in this study was 30.21%; 7.6% and 22.6% quarters showed clinical and subclinical mastitis, respectively. The analysis showed that statistically significant association with the risk factors namely breed, milking hygiene and teat lesion ($p<0.05$) with mastitis. In this study, only two bacterial species such as *Staphylococcus aureus* (57.3%) and *Escherichia coli* (42.7%) were isolated. In conclusion, the present study revealed the prevalence and the associated risk factors with bovine mastitis in dairy cows found in and around Assosa town. Therefore, appropriate mastitis control strategies should be implemented with consideration of the associated risk factors.

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1. Introduction

Ethiopia get economic benefit and social importance both at the household and national levels as well as significant export earnings in the past from the livestock's (Gebre Mariam *et al*., 2013). Among livestock, cattle take the majority with an estimated population of 59.5 million heads of cattle (CSA, 2016) which is the largest in Africa. From this cows represent the biggest portion of cattle population of the country, around 42% of the total cattle heads are milking cows. Despite the large number of dairy cows, milk production does not satisfy the nations' demand for milk due to a multitude of factors. From the factors animal diseases cause serious problem, particularly mastitis is one of the most economically important disease of the dairy industry (Biffa *et al.,* 2005).

Mastitis is a complex disease caused by a variety of microorganisms including bacteria, fungi and algae. But bacterial pathogen including Staphylococci, Streptococci, and Enterobacteriacae take the majority of the infections and coagulase-negative Staphylococci, environmental Streptococci, *Mycoplasma species*, and *Serratia spp.* are increasingly implicated as emerging pathogens causing mastitis such as (Sarba and Tola, 2017).

Mastitis can be classified as clinical and subclinical based on their clinical sign. Clinical form of mastitis is manifested by morphological change in the udder and chemical as well as physical changes in the milk and the subclinical form of mastitis is happened without any visible manifestations of inflammation and more common than the clinical mastitis with causing greatest economic loss in most dairy herds. Subclinical mastitis can be examined indirectly by various techniques such as California mastitis test (CMT), the Modified White Side test (MWT), Somatic Cell Count (SCC), pH, and catalase tests. These tests are preferable screening tests for subclinical mastitis as they can be used easily, rapid yielding and satisfied results (Sarba and Tola, 2017).

Mastitis can be considered as welfare, food safety and economic problem (Idriss *et al.,* 2013. It imposes economic losses through affecting animal health, reduction in milk production, culling of the diseased animals, cost of veterinary care and it has also public health importance by serving as a vehicle in the spread of diseases like tuberculosis, staphylococcal food poising and brucellosis (Radostits *et al.*, 2007). Most estimates show that on the average affected quarter suffer a 30% reduction in productivity and affected cow is estimated to lose a 15% of its production of cow per lactation (Blowey *et al*., 2010). According to the report of Moungube *et al.* (2005) both clinical and subclinical mastitis cause loss of 270 ETB per lactation in Ethiopia.

In Ethiopia, based on CMT, SCC and bacteriological investigation, the prevalence rate for all types of mastitis in dairy cows was ranged from 0.4%-81.1% in different parts of the country (Ismael, 2018). But in some parts of the country, including the Assosa town, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is poor. In this town there is growth of modern small holder dairy farms using cross breeds as well as local breeds of cattle to supply the high demand of milk for the fast growth of the human population in the town. More over there is no stable cattle population in the town due to transportation and exchange of cattle between the neighboring regions and countries as well as with in the region. For this reason conducting study on reproductive health problems particularly on mastitis is important to improve the dairy production. Therefore the objectives of this study were:-

- To estimate the prevalence of bovine mastitis in and around Asosa town
- To assess the potential risk factors associated with bovine mastitis
- To isolate and identify *S. aures* and *E. coli* from mastitic milk

2. Material and Methods

2.1. Study Area

The study was conducted from November 2018 to May 2019 in and around Asosa town which is the capital city of Benishangul Gumz Regional State and found 687 kms Northwest of Addis Ababa. It's located at 8°30'and 40°27' N latitude and 34°21' and 39°1' E longitude. The altitude of Asossa ranges from 580 to over 1560 meter above sea level. This area is characterized by low land plane agro-ecology with average annual rainfall of 850-1200 mm with unimodal type of rainfall that occurs between April and October (NMSA, 2014). Its annual temperature ranges between 18°C and 30°C. Asosa zone has 35.6% of the livestock population of the region constituting 61, 234 cattle, 191, 83 goats, 19,729 sheep, 25,137 donkeys, 439,969 poultry and 73,495 beehives (CSA, 2015).

2.2. Study Population

The study population were dairy cows of both breeds namely cross breed (Holstein-Friesian-zebu crosses) and local zebu breeds found in and around Asosa town. Age of the animal was determined based on the owner information and dental eruption. Dairy cows were categorized as young (3-≤6), adult (>6- ≤9) and old (>9).

2.3. Study Design

Cross-sectional study was conducted in and around Asosa town from November 2018 to June 2019 to determine the overall prevalence of bovine mastitis and its associated risk factors in cross breed (Holstein-Friesian-zebu crosses) and local zebu breeds of dairy cows categorized based on their age, breed, parity, stage of lactation and milking hygiene.

2.4. Sampling Method and Sample size Determination

Purposive sampling method was used based on willingness of the animal owner to sample his animal and only animals of those owners who sold the milk to the community were selected. The sample size was determined by using the formula given by (Thrusfield, 2005), with 95% confidence level, 5% desired absolute precision and expected prevalence of 39.32% (Tassew and Legesse, 2017).

$$n = \frac{(1.96)^2 p_{exp}(1-p_{exp})}{d^2} = \frac{(1.96)^2 0.52(1-0.52)}{0.05^2}$$

Where; n= required sample size

P_{exp}= expected prevalence

d^2= desired absolute precision (0.05)

Therefore, by using the expected prevalence of 39.32% from Benishangul-Gumuz region, the number of study animals examined in this study was 367 dairy cows.

2.5. Study Method

2.5.1. Examination of the udder

Examination for the presence of any abnormalities was done by visual inspection and palpation of the Udders of the cows. In addition, any change in color and consistency of milk was done by withdrawn the milk from each quarter. Based on this, a cow with observable clinical signs was recorded at the time of milk sampling. Theses clinical signs include manifestation of visible signs like inflammation of udder characterized by warm and swollen with painful upon palpation and gross changes in milk was well considered otherwise chronic mastitis when misshaped, atrophied, hard and fibrotic quarters were examined (Quinn *et al*., 2002).

2.5.2. Sample collection

Before collection of milk samples the udder, teat orifice and hands of the milkers were perfectly cleaned with water and soap and disinfected with 70% ethanol. The first streams of milk were discarded and about 20 ml of milk were collected in clean sterile capped bottle, each bottle was coded. The collected milk samples were properly labeled and immediately transported to Asosa Regional Veterinary Laboratory in an ice box with freeze packs under sterile conditions for microbiological analysis (Quinn *et al*., 2002).

2.5.3. California mastitis test (CMT)

California mastitis test (CMT) recommended by Quinn *et al*. (2002) was carried out for diagnosis of subclinical mastitis. Briefly, from each quarter of the udder, 2ml milk sample was dropped in each of the strip cups on the CMT paddle and an equal amount of 3% CMT reagent is added to each cup and mixed gently. The result was interpreted based on the thickness of gel formed by CMT reagent and milk mixture and scored as 0(negative), T (trace), 1(weak positive), 2(distinct positive) and 3(strong positive). Finally quarters with CMT score of 1 or above were identified as positive for sub clinical mastitis and 0 and T were recommended as negative (Quinn *et al*., 2002).

2.5.4. Bacteriological examination

Milk samples from positive for either clinical and sub clinical mastitis cows was bacteriologically examined according to Quinn *et al*. (2002). Briefly from each infected quarter, a loop full of mastitic milk sample was taken and inoculated separately on Mannitol salt agar and MacConKey's agar and incubated aerobically at 37°c for 24 to 48 hours. Then the inoculated plates were recorded after they were observed for presence or absence of bacterial growth, colony morphology and their color on both agars. Bacterial colonies was again sub-cultured on nutrient agar for further identification by gram staining and biochemical confirmation (such as catalase, oxidase, indole, and methyl red test) as described in Table 1 below.

Test and staining	*S. aures*	*E. coli*
Gram staining	+	-
KOH	-	+
Catalase	+	+
Coagulase	+	-
Oxidase	-	-
Hemolysis	+	-
Indole	-	+
Methyl red test	+	+
VP	-	-
Citrate utilization	-	-
Motility	-	+
XLD	-	-
Manitol salt agar	+	-
EMB	-	+
TSI gas	-	+
TSI slant	+	+
TSI butt	+	+
TSI H2S	-	-

KOH= Potassium hydroxide, EMB=eosin methylene blue, XLD=xylose lysine desoxychocolate agar, VP = voges proskaeure, TSI=triple sugar iron. Source: Quinn *et al*. (2002).

2.6. Data Analysis

The data derived from the study was coded properly and entered into Microsoft excel spread sheet 2013. Then the data was exported and analyzed by using STATA12. Chi-square and multivariate logistic regression and 95% confidence level was used to assess the association of potential risk factors with the prevalence of mastitis. Risk factors was considered significant when $p<0.05$.

3. Results

3.1. Overall Prevalence of Mastitis

Among the total 367 dairy cows examined for bovine mastitis, 127 were found positive, making the overall prevalence of 34.6% (127/367) in dairy farms found in and around Asosa town. Out of these, 7.9% (29/367) and 26.7% (98/367) were clinical mastitis and subclinical mastitis cases, respectively. Totally, 1468 quarters of udder were examined; of these 96.3% (1413/1468) milk samples were collected from non-blind teats, while the rest 3.7% (55/1468) teats were blinded. The quarter level prevalence of mastitis was 30.21% (427/1413). Out of the total quarters 7.6% (108/1413) quarters and 22.6% (319/1413) quarters were showed clinical and subclinical mastitis, respectively. Cow and quarter level prevalence result of clinical and sub clinical mastitis is listed in the Table 2.

Table 2: Cow level and quarter level prevalence of mastitis in the dairy cows

Type of mastitis	N° examined	N° positive	Prevalence (%)
Clinical mastitis			
Cow level	367	29	7.9
Quarter level	1413	108	7.6
Subclinical mastitis			
Cow level	367	98	26.7
Quarter level	1413	319	22.6

3.2. Association of Risk Factors with Mastitis

As shown in the table 3 below, risk factors such as age, breed, parity, stage of lactation, previous mastitis history, milking hygiene and teat lesion was considered in this study. The analysis showed statistically significant difference among breeds

($p<0.05$) with the occurrence of bovine mastitis in cross breeds (47.05%) than local breeds (25.7%). There was also statistical significant association ($p<0.05$) in milking hygiene and in dairy cow's teat lesion, with prevalence rate of 39.07% in poor milking hygiene and 66.67% in cow's with teat lesion respectively.

On the other hand, this study showed insignificant association of mastitis ($p>0.05$) with age, parity, stage of lactation and previous mastitis history. Even though these risk factors did not show statistically significant association, there was prevalence different among the different categories of the risk factors. From age categories young cattle's had higher prevalence (42.1%) than old (34.61%) and adult (29.9%) dairy cows, respectively. Cows with many calves (36.14%) show higher prevalence than cows with few calves (34.15%). Late (38.20%) lactation stage showed highest prevalence followed by dry (37.03%), early (34.71%) and mid (27.63%) stage of lactation. With respect to previous mastitis history, cow's having previous mastitis showed more prevalence of the disease than cow's not infected previously as described in table 3 below.

Table 3: Prevalence of mastitis on cow level and associated risk factors

Factor	Categories	Total no examined	NO of positives (%)	OR	x^2	P-value	95%CI LC	UC
Age	3≤6(young)	133	56(42.1)		5.421	0.065		
	>6≤9(adult)	208	62(29.9)	1.418			0.9789	2.055
	>9(old)	26	9(34.61)					
Breed	Zebu	214	55(25.7)		17.983			
	Cross	153	72(47.05)	0.389		0.000	0.250	0.605
Parity	1-4(few)	284	97(34.15)		0.1124			
	≥5(many)	83	30(36.14)	0.916		0.738	0.549	1.526
Stage of lactation	Early	121	42 (34.71)		2.3544			
	Mid	76	21 (27.63)	0.938		0.505	0.778	1.130
	Late	89	34 (38.20)					
	Dry	81	30 (37.03)					
Previous mastitis history	Infected	37	17(45.94)		2.338			
	Non-infected	330	110(33.333)	1.7		0.129	0.856	3.375
Milking hygiene	Poor	238	93(39.07)		5.980			
	Good	129	34(26.37)	1.792		0.015	1.119	2.868
Teat lesion	Yes	21	14(66.67)		10.118			
	No	346	113(32.67)	4.123		0.003	1.619	10.501

CI= Confidence Interval; OR= Odds Ratio; LC= Lower Class; UC= Upper Class

3.3. Bacteriological Examination Result

Among 427 clinical and CMT positive milk samples, 36.76% (157/427) milk samples show growth on Mannitol salt agar with catalase (+), coagulase (+), oxidase (-), hemolysisi (+), indole (-) and methyl red test (+) and also show growth on MacConkey agar with EMB (+), catalase (+), oxidase (-), indole (+), methyl red tes (+), VP (+),. TSI gas (+), TSI slant (+), TSI butt (+) and TSI H2S (-). But the remaining did not show growth on these media. Of the total culture positive samples, such as gram positive *S. aureus* 57.3% (90/157) and gram negative *E.coli* 42.7% (67/157), were isolated, Table 4.

Table 4: Bacterial isolates and their prevalence

Microorganism	Clinical	Subclinical	Total	Percentage
S. aures	34	56	90	57.3%
E.coli	23	44	67	42.7%
Total	67	90	157	100%

4. Discussions

The profitability of dairy industry is significantly affected by mastitis. It's a multi-factorial disease

which influenced by combination of environmental and pathogenic factors and with variable responses between animals. Identification of risk factors for this type of disease is important for the development of control and prevention strategies (Bastan *et al*., 2013). The overall prevalence of mastitis in the current study was 34.6% (127/367). This result show is in agreement with previous findings of Biffa *et al.,* (2005) in Southern Ethiopia who reported 34.9% overall prevalence of mastitis. But it's lower than the finding of Tassew and Legesse (2017) which was 39.32% conducted on the same area and much lower than the reports of Zelalem (2017) in Lemmo wereda, southern Ethiopia, Yomiyu *et al.* (2017) in Sebeta, Tesfaye and Abera (2018) in Jimaa which was 53.3%, 56.5% and 62.96% respectively. This level is higher than report of Nessru (1997) which was 25%. The variation in the reported in different studies might be due to different factors which may include difference in management system, breed considered, level of production, unhygienic housing, deficient in milking procedures, poor milking hygiene of the cows (Kivaria *et al*., 2004).

The occurrence of subclinical mastitis in this study is higher than clinical mastitis which is supported by the previous studies which indicated that subclinical mastitis is 3-4 times more frequent than clinical mastitis (Radiostitis *et al.*, 2007). This is also further supported by many studies performed in Ethiopia like the reports of Yomiyu *et al*. (2017) in Sebeta with 9.31% (27/290) and 47.24% (137/290), Sarba and Tola (2017) in Ambo district with 9.9% (30/302) and 32.8% (96/302), Tesfaye and Abera, (2018) in Jimma town with 2.3% and 60.65% for clinical and sub clinical mastitis respectively. The lower prevalence of clinical mastitis may be attributed to the treatment of cows after manifesting clinical sign of mastitis. The higher prevalence of sub clinical mastitis may be due to strong cow's defense mechanism to minimize the severity of the disease (Jha *et al.,* 2010) and further more farmers in the study area lack awareness and little attention is given for sub clinical mastitis which made easy to transmit it from the mastitic cows to healthy cows.

This study indicated that more prevalence of mastitis in cross breeds (47.05%) than local breeds (25.7%). This finding is also further supported by the finding of Tassew and Legesse (2017) in and around Assosa town which indicated that breed has significant on the occurrence of mastitis ($p<0.05$) with 50.86% in cross breed and 34.32% in local zebu breeds. This may contributed by impact of genetic traits on the susceptibility of the animal to mastitis include the natural resistance, milk yield, teat shape and conformation, positioning of udders and relative distance between teats. (Radiostitis *et al.,* 2007).

Milking hygiene has statistically significant association with the occurrence of mastitis ($p<0.05$). Cows under poor milking hygiene (39.07%) have high infection rate than cows under good hygiene (26.37%). This is may be due to lack of hand washing of the milker before, during and after milking, washing hand between the milking, udder and teat washing before milking. Teat lesions also showed significant association ($P< 0.05$) with more mastitis prevalent in cow with teat lesion (66.67%) than cow without teat lesion (32.67%). This finding is supported by the findings reported of Sori *et al.* (2005) with the prevalence of 68.8% in cow with teat lesion and 18.2% in cow without lesion and by Biffa *et al.* (2005) with prevalence of 84% in cow with teat lesion and 47.7% in cows without teat lesion. Animals with skin lesions on the teat had a high prevalence of mastitis probably due to lesions of the skin which is the first defense mechanism and protective barrier against pathogen invasion.

The bacteriological finding indicated that contagious *S. aureus* bacteria was the predominant followed by environmental mastitis of *E. coli* with 57.3% (90/157) and 42.7% (67/157) respectively. The finding of *Staph. aures* is slightly lower than the report of Gemechu *et al.* (2019) which was 59.26% in Bench Maji zone, Southwest Ethiopia and higher than the report of Fufa *et al.* (2013) which was 21.13% in Addis Ababa city and Bitew *et al.* (2010) which was 20.3% in Bahir Dar. But it have close agreement with the previous report of Endale *et al.* (2016) who reported that 57.14% in and around Sodo Town, Wolaita Zone, Ethiopia. The highest isolation rate of the *S. aureus* might be due to wide distribution of organism inside the mammary gland and on the skin of the tests and udder, lack of effective hand udder and teat washing and drying, lack of proper milking procedure and hygiene before milking, during milking and post milking in areas where hand milking has been practiced.

The finding of *E.coli* is higher than the previous reports of Tola, (1996) which was 34.9% in Arsi. But it's comparable with the report of Biruke and Shimeles (2015) which was 40.7%, in Addis Abeba. The justification for the high isolation rate of *E.coli* is might be due to implication of unhygienic milking practice and contamination of cows' teats and environment with their dung in the study area attributed to the occurrence of mastitis due to environmental pathogen.

5. Conclusion and Recommendations

The present study indicated that mastitis is prevalent in the dairy cow in and around Assosa town with subclinical mastitis is more frequent than clinical mastitis. Breed, milking hygiene and teat lesion was

the risk factors associated with the disease. In the study area mastitis caused by *S. aures* was the major problem in the dairy cows than *E. coli*. This implied that mastitis have an economic impact on the dairy production and food security in the area. Therefore, based on the above conclusion the following recommendations are forwarded:-

- Mastitis control strategies should be implemented with consideration of the associated risk factors.
- Extensions packages that increase farmer's awareness on subclinical mastitis would be implemented in mastitis control and improve farmers' income.
- Hygienic milking practice and hygiene, appropriate milking procedure, environmental hygiene as well as the cow's udder and teat hygiene should be kept to decrease contagious and environmental mastitis.

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