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Prevalence, risk factors and major bacterial causes of bovine mastitis in and around Wolaita Sodo, Southern Ethiopia

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A cross-sectional study was conducted from November 2011 to March 2012 on lactating dairy cows to determine the overall prevalence of bovine mastitis, identify associated risk factors and isolate the predominant bacterial agents involved in causing mastitis in and around Wolaita Sodo. A total of 349 lactating cows were examined for mastitis using clinical examination and California mastitis test (CMT). Bacteriological methods were also employed to isolate the causative bacteria. An overall 29.5% (95% CI: 24.7, 34.3) prevalence of mastitis was recorded in the area of which 2.6% (95% CI: 0.9, 4.3) were clinical and 26.9% (95% CI: 22.2, 31.6) subclinical cases. About 90 bacterial isolates belonging to 6 species were identified from mastitic milk samples. The isolates based on their relative frequency of occurrence were *Staphylococcus aureus* (30%), *Streptococcus agalactiae* (17.78%), *Escherichia coli* (17.78%), *Staphylococcus epidermids* (13.3%), *Corynebacterium bovis* (12.2%) and *Streptococcus dysgalactiae* (8.9%). The prevalence of mastitis varied significantly (p<0.05) among breeds, where the highest prevalence was recorded in Jersey (60% (95% CI: 49.3, 70.7)), followed by Holstein-Zebu cross (30.8% (95% CI: 22.9, 38.7)) and Zebu (10.8% (95% CI: 5.6, 16.0)). It was also appreciated that lactation stage and parity significantly (p<0.05) influenced the occurrence of mastitis. Animals at early stage of lactation were severely affected with mastitis than at end and mid lactation stages. The prevalence of mastitis was found to have a direct relation with parity, as the occurrence of mastitis increased with parity. Inadequate sanitation of dairy environment and lack of proper attention to health of mammary gland were important factors (P<0.05) contributing to the prevalence of mastitis. Generally, the study showed that mastitis is an important problem and a serious threat for dairy industry in the study area. Therefore, appropriate control measures targeting the specific causative agents should be in place to reduce the impact of the disease. The farmers should also be aware of the impact of the disease and practice hygienic milking, culling of chronic mastitis carriers and treating of clinically infected cows.

Key words: Bacterial isolates, bovine mastitis, lactating cow, prevalence, risk factors, Wolaita Sodo.

INTRODUCTION

Ethiopia, located in tropical region, is one of the most populous countries in Africa, having an estimated population of more than 80 million. The country is very much dependent on agriculture. Livestock represent a major national resource and form an integral part of the agricultural production system. Ethiopia has the largest cattle population in Africa with an estimated population of 49.3 million. Cow represents the biggest portion of cattle population of the country, around 42% of the total cattle heads are milking cows (CSA, 2008). However, milk

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production often does not satisfy the country's requirements due to a multitude of factors. Mastitis is among the various factors contributing to reduced milk production (Biffa et al., 2005).

Mastitis is an inflammation of the mammary gland that can be caused by physical or chemical agents but the majority of the causes are infectious and usually caused by bacteria (Quinn et al., 2002; Radostits et al., 2007). Over 140 different microorganisms have been isolated from bovine intramammary infection, but the majority of infections are caused by *Staphylococci*, *Streptococci* and *Enterobacteriacae* (Bradley, 2002; Radostits et al., 2007). Mastitis is the most important and expensive disease of dairy industry. It results in severe economic losses from reduced milk production, treatment cost, increased labour, milk withheld following treatment and premature culling (Miller et al., 1993).

In view of the degree of inflammation, mastitis can be classified as clinical and sub clinical types (Philpot and Nickerson, 1991). Clinical mastitis includes gross abnormality in milk, physical abnormalities of udder and abnormality of cow with systemic involvement. Sub clinical mastitis is characterized by absence of gross lesion and an increase in number of somatic cell in the milk. It is more prevalent than clinical mastitis and with long duration, reduced production and affect quality of milk produced (Philpot and Nickerson, 1991; Radostits et al., 2007). According to Radostits et al. (2007), the diagnosis of bovine mastitis is performed by clinical examination (inspection and palpation) for clinical forms of mastitis, screening (CMT) test for subclinical forms of mastitis and bacterial isolation for confirmatory diagnosis.

In Ethiopia, mastitis has long been known (Biffa et al. 2005; Tamirat, 2007; Almaw et al., 2009; Bitew et al., 2010), however, the information on the magnitude, risk factors and causative agent of the disease is inadequate. Such information is important when designing appropriate strategies that would help to reduce its prevalence and effects (Biffa et al., 2005). Most studies in Ethiopia were carried out in Addis Ababa and its surroundings, which is not representative of other regions of the country (Almaw et al., 2009). In southern regional state, mastitis is not well considered. There is very few published material on the current status of mastitis in and around Wolaita Sodo (Tamirat, 2007). Hence this study was initiated with the objectives of determining the prevalence of bovine mastitis at Wolaita Sodo and its surroundings, assessing the associated risk factors and isolating the frequent bacterial causes.

MATERIALS AND METHODS

Study area

The study was conducted in and around Wolaita Sodo, Southern Nation Nationalities People Regional State, southern Ethiopia. Wolaita Sodo is located about 390 km south of Addis Ababa. The town Sodo is located at latitude of 8°50°N and longitude of 37°45°E.

Topographically, the area is marked by hilly, flat, steep slopes and gorges and a number of streams and mountains. The highest mountain is Damota, 2500 m above sea level, which is located near Sodo town (Tamirat, 2007). The Altitude varies from 1100-2950 m.a.s.l. The area experiences mean annual temperature of about 20°C. The mean maximum temperature is 26.2°C and the average monthly minimum temperature is 11.4°C.The rainfall regimes over much of the area are typically bimodal with the big rainy season extending from June to September and a small rainy season occurring from February to April. The mean annual rain fall of the area ranges from 450-1446 mm with the lowest being in low land and highest in high land. The livestock population in the area is estimated to be 68,900 cattle, 1992 sheep, 382 goats, 121 horses, 131 mules, 488 donkeys and 55,191 chicken (Wolaita Zone Agricultural Office, 2011).

Study animals and management systems

The study populations were all lactating cows in and around Wolaita Sodo. Lactating dairy cattle (most of the time indigenous zebu cattle from surrounding areas of Sodo) visiting Sodo zuria veterinary clinic, Jersey from Wolaita Sodo dairy farm and Holstein-Zebu cross from the small holder dairy farm in Sodo town constitutes the study animals. Individual animal was selected randomly and tested for mastitis using CMT and clinical examinations.

The indigenous zebu found in the study area is managed under extensive system as a source of milk, meat and drought power. The dairy herd of Jersey breed at Sodo farm is managed more or less semi intensively under the supervision of trained personnel. Small holder dairy farms in the town are with Holstein-Zebu cross mainly and they are housed always and provided feed in their stall.

Study design

A cross-sectional study was conducted from November 2011 to March 2012 in Wolaita Sodo and its surroundings, to determine prevalence, associated risk factors, and to isolate causative agents of bovine mastitis. Sample size was determined according to Thrusfield (2005) at 95% confidence interval, 5% precision and with expected prevalence of 34.9% (Biffa et al., 2005). A total of 349 lactating cows; 130 Holstein-Zebu cross, 80 Jersey and 139 Zebu were sampled. Individual animals were selected using simple random sampling method.

Cows were examined clinically and tested for mastitis screening with CMT, and cases found clinically mastitic or screening positive were sampled for bacterial isolation. The risk factors considered at the study were, age, parity, lactation stage, breed and hygienic milking. Age of the cows was determined by observing their dentition characteristics and grouped into < 5 years, 5-8 years and > 8 years category. Parity was categorized into 1-2 calves, 3-6 calves and > 6 calves. Milking hygiene practice was grouped into good (If there is a practice of washing and drying udder with separate towels, milking healthy and young cows first) and poor (If washing and drying of udder with a separate towel and milking with order is not practiced). Lactation stage of the cow was also categorized into early stage lactation (1-4 months), mid lactation (> 4 – 8 months) and late lactation (above 8 months).

Milk sample collection

Procedure for collection of milk was according to Quinn et al. (2002); strict aseptic procedures were adopted when collecting milk samples in order to prevent contamination with microorganisms present on the body of animal and from the barn environment. Milk

sampling and screening were performed for each quarter. The time chosen for sample collection was before milking. Information on the cow age, parity, breed, lactation stage and milking hygiene were also collected at the time of sampling using data recording sheet.

Diagnosis of mastitis

In the present study, the lactating cow's udder and teats were clinically examined by palpation to know the abnormalities before the collection of milk samples. According to Radostits et al. (2007), quarters revealing the following abnormalities were diagnosed as clinical mastitis; observation of abnormal milk with no visible and palpable changes in quarters, observation of abnormal milk with visible and palpable changes in quarters and acute mastitis with systemic involvement. If one of the above symptoms was observed, milk was sampled directly for bacterial isolation. Animals or cases not showing either signs were tested for screening with CMT test; those positive were sampled to perform bacterial isolation. Sampling only positive quarters may underestimate the prevalence of subclinical mastitis; however, this can be taken as a limitation of this study.

Microbiological procedures

Samples from CMT positive and from clinical mastitic cows were analyzed microbiologically based on Quinn et al. (1999, 2002) as absolute diagnosis and identification of the disease is based on isolation and identification of bacteria. Culturing of the collected samples was performed after centrifugation to concentrate the organisms then it was inoculated into blood agar medium. Mac-Conkey agar plate is streaked in parallel to detect gram negative bacteria. Edwards's media was used as selective for streptococcal organisms and for determination of hemolysis and aesculin hydrolysis. The inoculated plates were incubated aerobically for 24 to 48 h at 37°C. The result was declared as negative, if growth did not occur for 72 h of incubation.

Bacterial isolates were identified on the basis of colony characteristics, presence of haemolysis, Gram stain and biochemical tests. Coagulase test and maltos fermentation test (Quinn et al., 2002) were employed to differentiate *Staphylococcus aureus* from other staphylococcus species. Growth on MacConkey agar, aesculin hydrolysis on Edwards medium and CAMP test were also used to differentiate *Streptococcus agalactiae* from other mastitis causing streptococci.

Statistical analysis

Collected data were first entered into a Microsoft Excel spreadsheet and analyzed using Stata 11 software. Descriptive statistical analysis was used to summarize and present the data collected. The prevalence of mastitis was calculated as the number of lactating cows tested positive by CMT test or animals showing symptoms of clinical mastitis, divided by the total number of tested or clinically examined animals. The existence of association between the risk factors (age, parity, breed, lactation stage and milking hygiene) and mastitis was assessed using the Pearson Chi-square (χ^2) test. Besides, the degree of association between the risk factors and occurrence of mastitis were analyzed first with univariate logistic regression and those factors having a P-value less than 0.25 were further considered in multiple logistic regression analysis in the final model. The presence of interaction and adjustment for the confounding variables was not considered. Significant values were considered at P<0.05.

RESULTS

Prevalence of bovine mastitis

From the total of 349 lactating cows examined, 103 (29.5% (95% CI: 24.7, 34.3)) were positive for mastitis. Of these, 9 (2.6% (95% CI: 0.9, 4.3)) and 94 (26.9% (95% CI: 22.2, 31.6)) were found to be positive for clinical mastitis and subclinical mastitis, respectively. The results of bacterial cultures also revealed that 90 (87.4%) of the affected cows were with bacterial isolates and the rest 13 (12.6%) were without bacterial isolates. The study also considered mastitis at quarter level. Of 1396 quarters examined, 250 (17.9%) were found to be mastitis positive and 77 (5.5%) blind.

Risk factors

Among the five potential risk factors considered for a univariate logistic regression, all the risk factors age, parity, breed, lactation stage and milking hygiene were found significant (P<0.05) (Table 1). However, age became insignificant when tested with multivariate logistic regression (Table 2).

Animals over 8 years old were more frequently affected with the disease and those younger than 5 years were rarely affected (Table 1).The highest prevalence of mastitis was observed in Jersey (60%), followed by Holstein-Zebu cross (30.8%) and local Zebu (10.8%) (Table 1). The occurrence of mastitis was more than two times (OR (odds ratio) = 2.51) higher in Jersey than Zebu breeds. The difference among the three breeds was statistically significant (P<0.05) (Tables 1 and 2). Both parity and milking hygiene were found to be significantly (P<0.05) associated with the occurrence of mastitis. The occurrence of mastitis was higher in poor milking hygiene and lower at good milking hygiene. The highest prevalence of mastitis was observed in animals with parity of more than 6, followed by 3-6 and 1-2 parity (Tables 1 and 2). The occurrence of mastitis was about five times (OR = 4.83) more likely in animals with parity of more than six. Furthermore, mastitis prevalence was found to be higher in early lactation and lower in mid lactation stages (Table 1). Statistical analysis showed the existence of significant (P<0.05) association between the occurrence of mastitis and lactation stage (Table 2).

Generally, in this study, breed, parity, lactation stage and milking hygiene were considered as risk factors for the occurrence of mastitis and they were found significantly associated with the occurrence of mastitis (Table 2).

Bacterial isolation

Analysis of bacteriological examination of milk samples

Table 1. Univariate logistic regression analysis of risk factors for the occurrence of bovine mastitis in and around Wolaita Sodo, Southern Ethiopia.

Variable	Category	Number of cows examined	Number of cows with mastitis	Proportion of cows with mastitis (95% CI)	P-value	Odds ratio (OR) (95 % CI of OR)
Breed	Holstein-Zebu cross	130	40	30.8 (22.9-38.7)	0.000	1.66(1.26 - 2.18)
	Jersey	80	48	60.0 (49.3-70.7)		
	Zebu	139	15	10.8 (5.6-16.0)		
Age	<5 years	104	11	10.6 (4.7-16.5)	0.000	3.06 (2.14- 4.37)
	5 - 8 years	155	44	28.4 (21.3-35.5)		
	>8 years	90	48	53.3 (43.0-63.6)		
Milking hygiene	Good	159	20	12.6 (7.4-17.8)	0.000	0.19(0.11-0.32)
	Poor	190	83	43.7 (36.6-50.8)		
Parity	1-2	177	21	11.9 (7.1-16.7)	0.000	4.39 (2.93- 6.56)
	3-6	137	58	42.3 (34.0-50.6)		
	> 6	35	24	68.6 (53.2-84.0)		
Lactation stage	Early	94	62	65.9 (56.3-75.5)	0.000	0.44 (0.32-0.60)
	Mid	121	4	3.3 (0.1-6.5)		
	End	134	37	27.6 (20.0-35.2)		

Table 2. Multivariate logistic regression analysis of risk factors for the occurrence of bovine mastitis in and around Wolaita Sodo, Southern Ethiopia.

Variable	P-value	Odds ratio	(95 % CI)
Breed	0.000	2.51	(1.71-3.68)
Milking hygiene	0.000	0.14	(0.01-0.27)
Parity	0.000	4.83	(2.42-9.62)
Lactation stage	0.000	0.52	(0.36- 0.74)

Table 3. The frequency of bacteria isolated from bovine mastitis in and around Wolaita Sodo, Southern Ethiopia.

Organisms	Clinical mastitis	Sub clinical mastitis	Total	Relative frequency (%)
S. aureus	6	21	27	30.0
S. epidermids	-	12	12	13.3
Str. agalactiae	-	16	16	17.8
Str. dysgalactiae	-	8	8	8.9
C. bovis	-	11	11	12.2
E. coli	3	13	16	17.8
Total	9	81	90	100

was made to identify the main etiological agents involved in the disease. The organisms were identified on the basis of their cultural, staining characteristics and biochemical reactions. In the study period, about 6 bacterial species and 90 bacterial isolates: *S. aureus,* 27 (30.0%), *Str. agalactiae*, 16 (17.8%), *E. coli*, 16 (17.8%), *S. epidermids*, 12 (13.3%), *C. bovis*, 11 (12.2%) and *Str. dysgalactiae*, 8 (8.9%) were observed (Table 3).

DISCUSSION

The study was conducted on bovine mastitis in Wolaita Sodo and its surroundings to determine the prevalence and major risk factors associated with the disease. The result revealed an overall prevalence of 29.5% (955 CI: 24.7, 34.3) in the study area. This result agrees with the previous report by Bitew et al. (2010), who recorded an

overall prevalence of 28.2% at Bahir Dar and its environs. The current finding of the study is slightly lower than that of Nesru (1999) and Biffa et al .(2005), who reported an overall prevalence of 37.2% in the urban and peri-urban dairy farms at Addis Ababa, central Ethiopia and 34.9% in the southern Ethiopia, respectively. It is comparably very low when compared with the work of Sori et al. (2005) and Lakew et al. (2009), who reported a prevalence of 52.78% in and around Sebeta and 64.4% in Asella, respectively. Mastitis is a complex disease and the difference in results could be due to difference in management system of the farm, the breeds of cattle considered and the geographical locations of the studies.

The prevalence of clinical mastitis recorded in the present study is 2.6% (95% CI: 0.9, 4.3) and that of subclinical mastitis 26.9% (955 CI: 22.2, 31.6) which is by far higher than the occurrence of clinical cases. This finding is in line with the report of Bitew et al. (2010), who reportted clinical prevalence of 3% and also subclinical cases of 25.2% at Bahir Dar and its environs. However, it is lower than that from the findings of Nesru (1999), who recorded 5% clinical and 32.2% sub clinical cases in the urban and peri-urban dairy farms at Addis Ababa, central Ethiopia. The results of both clinical and subclinical mastitis in this study are also incomparable with those previous reports in Ethiopia (Sori et al., 2005; Biffa et al., 2005; Lakew et al., 2009). In most reports including the present study, clinical mastitis is far lower than subclinical mastitis (Sori et al., 2005; Biffa et al., 2005; Almaw et al., 2008; Lakew et al., 2009; Haftu et al., 2012). This could be attributed to little attention given to subclinical mastitis, as the infected animal shows no obvious symptoms and secrets apparently normal milk and farmers, especially small holders, are not well informed about invisible loss from sub clinical mastitis. In Ethiopia, the subclinical forms of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Almaw et al., 2008).

The quarter level mastitis prevalence (17.9%) recorded in the current study is comparable with the finding of Haftu et al. (2012). The finding of 5.5% blind teat in this work is in agreement with the previous reports (Almaw et al., 2008; Lakew et al., 2009) and higher when compared with the report of Haftu et al. (2012) and Bitew et al. (2010). The blind quarters observed in this study might be an indication of a serious mastitis problem on the farms and of the absence of culling that should have served to remove a source of mammary pathogens for the cows.

Association of mastitis occurrence with parity was evaluated and found statistically significant (P<0.05). The increased prevalence of mastitis with parity reported in the current study is comparable with the previous reports (Biffa et al., 2005; Tamirat, 2007; Mekibib et al., 2010; Haftu et al., 2012). This might be due to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without mastitis con-

trol program (Radostits et al., 2007).

The occurrence of bovine mastitis and lactation stage was significantly (p<0.05) associated. That is, higher infection in cows in early lactation stage followed by late and medium lactation stages, that concurs with previous reports (Biffa et al., 2005; Tamirat, 2007). The early lactation stage infection might be due to the carryover of infection from dry period. In cows most new infections occur during the early part of the dry period and in the first two months of lactation (Radostits et al., 2007).

The study showed that breed significantly (P<0.05) influenced the occurrence of mastitis where Jerseys were severely affected than Holstein-Zebu cross or local Zebu. The report is comparable with findings of other studies such as Almaw et al. (2009) in Gondar town and its surroundings, Sori et al. (2005) in and around Sebeta and Junaidu et al. (2011) at Sokoto metropolis. However, mastitis occurrence among breeds might reflect the differences in management rather than a true genetic difference (Radostits et al., 2007). So, the higher susceptibility of Jerseys here might be the reflection of the farm hygiene and other management practices at Wolaita Sodo dairy farm.

Prevalence of mastitis was significantly (p<0.05) associated with milking hygienic practice. Cows at farms with poor milking hygiene standard are severely affected than those with good milking hygiene practices (Junaidu et al., 2011; Lakew et al., 2009; Sori et al., 2005). This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could be vectors of spread especially for contagious mastitis.

In the current study, six bacterial species were isolated: *S. aureus*, *S. epidermids*, *Str. dysgalactiae*, *Str. agalactiae*, *E. coli* and *C. bovis*. This report closely agrees with the reports of Sori et al. (2005), Lakew et al. (2009) and Bitew et al. (2010). Bacterial isolates were recorded from all 9 clinically affected cows. The isolates from these clinical cases were *S. aureus* and *E. coli*; this is in agreement with Mekibib et al. (2010), who reported all the 12 clinical cases which were culture positive at Holeta town, central Ethiopia.

S. aureus was the predominant pathogen involved in constituting 30% of all bacterial isolates in the current study. This concurs with Delelesse et al. (2010) but not with Atyabi et al. (2006) or Mekibib et al. (2010) which were lower and higher, respectively than the current study. The relative high prevalence of *S. aureus* in the current study shows the absence of dry cow therapy and low culling rate of chronically infected animals practice in the study area.

Streptococcus species were also found prevalent with 27.7% share of the total isolates: *Streptococcus agalactiae* 17.8% and *Streptococcus dysgalactiae* 8.9%. This finding coincides with that of Hawari and Al-dabbas (2008), who reported 26.2% relative frequency of *Streptococcus* species in Jordan. However, the finding is higher than the

reports of Bitew et al. (2010) at Bahir Dar and its environs (13.9%) and Sori et al. (2005) in and around Sebeta (3.73%) and lower than the report of Atyabi et al. (2006) at farms around Tehran (33.54%).

E. coli is occurred in higher extent than expected by contributing17.8% of the isolates. The findings is different from the previous reports by Mekibib et al. (2010) at Holeta (4.6%) and Sori et al. (2005) in and around Sebeta (0.75%), but agrees with the report of Hawari and Al-dabbas (2008) in Jordan (15.6%). *E. coli* is an environmental contaminant and its high prevalence in the present report could be related to hygienic status practiced at the study site, particularly at Wolaita Sodo dairy farm where the milking hygiene practice is very poor.

There are also other coagulase negative bacteria like *S. epidermids* which contributes about 13.3% of the isolates which is in line with the report of Sori et al. (2005) in and around Sebeta (14.93%). *C. bovis* was also isolated with a relative frequency of 12.2%. This differs from other reports (Sori et al., 2005; Mekibib et al., 2010; Junaidu et al., 2011).

In conclusion, this study revealed considerable prevalence of mastitis with the isolation of major pathogens such as *S. aureus, Str. agalactiae* and *E. coli* in and around Wolaita Sodo. Therefore, appropriate control measures targeting the specific causative agents should be in place to reduce the impact of the disease on dairy industry of the study area. The farmers should also be aware of the impact of the disease and practice hygienic milking, culling of chronic mastitis carriers and treating of clinically infected cows.

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