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Study on Bovine Mastitis in Dairy Farms of Bahir Dar and its Environs

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Abstract: Cross-sectional study was conducted on a total of 302 lactating (local and crossbred) dairy cows to determine the overall prevalence of mastitis and to isolate and identify the predominant bacterial agents involved in Bahir Dar town and its environs using Californian Mastitis Test (CMT) as screening tests. The overall mastitis prevalence recorded in the area was 85 (28.2%), out of which 9 (3%) clinical and 76 (25.2%) were subclinical cases. Of 1208 quarters examined, 23 (1.9%) were blind teats and 134 quarters (12.3%) showed evidence of infection of sub clinical mastitis. About 79 bacterial isolates were identified from CMT positive samples. Among the isolates were Coagulase Negative Staphylococci (CNS) (51.9%), *Staphylococcus aureus* (20.3%), *Streptococcus agalactiae* (8.8%), *Corynebacterium bovis* (0.75%) and *Bacillus* species (0.75%). Other species isolated include *Streptococcus dysagalactiae* (5.1%), *Micrococcus* species (3.8%), *Streptococcus uberis* and *Actinomyces pyogenes* (2.5%) each. The occurrence of mastitis varied significantly ($p<0.05$) between crossbred 61 (36.7%) and local Fogera 24 (17.6%). The prevalence of mastitis also significantly differed between animals with udder/or teat lesion, hygiene and parity number ($p<0.05$) in both breeds. There was no significant differences ($p>0.05$) between lactating cows with lactation stages in local Fogera breeds but it was significant ($p<0.05$) in crossbreds. The study showed that mastitis is the problem of dairy cows in the study area and the major isolates were contagious pathogens therefore, hygienic milking practice, culling of chronically infected cows and hygienic practices in the environment should be followed.

Key words: CMT, etiological agents, lactating cow, mastitis, prevalence, Bahir Dar, Ethiopia

INTRODUCTION

Mastitis is a disease of many mammalian species. At least, 137 infectious causes of bovine mastitis are known to date and in large animals the commonest pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, other *Streptococcus* species and Coliforms (Sumathi *et al*., 2008).

It may also be associated with many other organisms including *Actinomyces pyogenes*, *Pseudaomonas aeruginosa*, *Nocardia asteroides*, *Clostridium perfringens* and others like *Mycobacterium*, *Mycoplasma*, *Pastuerella* and *Prototheca* species and *yeasts* (Rodostits *et al*., 2007). The majority of the cases are caused by only a few common bacterial pathogens, namely, *Staphylococcus* species, *Streptococcus* species, Coliforms and *Actinomyces pyogenes* (Du Preeze, 2000; Quinn *et al*., 2004).

However, mastitis commonly seen as subclinical, clinical mastitis is also could be observed this might be manifested as per acute, acute or chronic forms (Radostits *et al*., 2007). According to Lemma *et al*. (2001) of the major diseases of crossbred cows in Addis Ababa milk shed mastitis was the second most frequent disease next to reproductive diseases. Mungube *et al*. (2005) and Tesfaye *et al*. (2010) estimated the economic losses from mastitis in the urban and periurban areas of Addis Ababa, to be US$ 58 and 78.65 per cow per lactation, respectively. The prevalence of clinical and subclinical mastitis in Ethiopia range from 1.2-21.5 and 19-46.6%, respectively (Hussein, 1999; Kassa *et al*.,1999; Lemma *et al*., 2001; Workineh *et al*., 2002; Dego and Tareke, 2003).

However, most of these studies were carried out in Addis Ababa and its surroundings, capital of the country and fail to represent the occurrence of mastitis under different management and environmental situations in other regions of the country. In Amhara regional state, mastitis is not a disease that is well considered, despite its economic and public health importance (Almaw *et al*., 2008, 2009).

There is few published data about the current status of mastitis in dairy farms of Bahir Dar town and its environs (Almaw *et al*., 2008) in which the research was undertaken in 2003. Therefore, the objectives of this study were to determine and compare the prevalence of mastitis in crossbred and indigenous local Fogera breeds of

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lactating cows in the study area to isolate and identify the predominant bacterial agents involved in the development of bovine mastitis and to determine the association of some potential risk factors.

MATERIALS AND METHODS

Study area: The study was conducted in dairy farms of Bahir Dar and its environs, western Gojjam Administrative zone. Geographically the region is located between 9°20 and 14°20 latitude North and 30°20 and 40°20 longitude East and covers an area of the country which estimated to be 170.052 km^2.

Bahir Dar is the capital city of Amhara region and is located at about 570 km North-West of Addis Ababa. It has a summer rainfall, the highest rainfall is between June and September and a winter dry season (December to March) with mean annual rainfall at 1200-1600 mm, mean temperature 10-20°C and an altitude at 150-2300 m above sea level (Bureau of Agriculture, 2006).

Study animals: The study populations were all lactating local Fogera and Holstein-zebu crossbred cows from dairy farms in and around Bahir Dar. The study was conducted on simple randomly selected 302 lactating dairy cows 136 indigenous Fogera and 166 Holstein-Zebu crosses from 37 small holder dairy farms in Bahirdar and Andassa Livestock Research Center. The average herd size was 7.9 and the maximum was 60 lactating cows of Andassa Livestock Research Center (ALRC).

Study design: Cross-sectional study and laboratory isolation of the bacteria were undertaken from November 2009 to April 2010 on dairy farms in Bahir Dar and its environs. Cows were examined directly at quarter level for Clinical Manifestations and Indirect Tests (CMT) for subclinical prevalence.

Sample size determination and sampling strategy: The sample size was calculated according to the formula given by Thrusfield (2005). It was calculated by taking 26.9% estimated prevalence from previous report by Almaw *et al.* (2008) and 95% confidence levels and 5% precision level. Simple random sampling method was considered to select the individual dairy cow.

Milk sample collection: Milk sample collection was according to the procedures recommended by National Mastitis Council (NMC, 1990). Duplicate quarter foremilk samples of approximately 10 mL amount was taken; one sample was used for CMT and the remaining sample used for bacterial isolation. After collection, samples were placed in icebox and processed in the same day or within few days. Teats towards sample collection were sampled first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was held as near horizontal as possible and by turning the teat to a near horizontal position, approximately 10 mL of milk was collected into a universal sample collection bottle.

CMT screening: The California Mastitis Test was carried out according to the method described by Quinn *et al.* (2004). A squirt of milk about 2 mL from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup.

A gentle circular motion was applied to the mixtures a horizontal plane for 15 sec. The result was scored from 0-3 (Quinn *et al.*, 2004).

Bacterial isolation: Bacteriological examination of the milk was carried out for CMT positive results following standard procedures (Quinn *et al.*, 2004). Milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature (25°C) for about an hour and then mixed by shaking. The samples were allowed to stand for a while for the foam to disperse and just before inoculation the tube was inverted gently. The samples were cultured and the primary and secondary bacterial identifications were done according to the method by Quinn *et al.* (2004).

Statistical analysis: Factors that affect the prevalence of mastitis included in this study were breed, stage of lactation, parity, hygiene and presence or absence of udder/or teat lesion. These factors were entered in to the MS Excel sheet and analyzed by using SPSS version 16.0 software and 95% was taken for significance level of the result. The Chi-square (χ^2) test was applied to test the existence of association between CMT positivity and the risk factors.

RESULTS AND DISCUSSION

Of the total 302 lactating cows examined overall mastitis prevalence in the area recorded was 85 (28.2%). Significant difference ($p<0.05$) was observed in prevalence of mastitis between local Fogera and Cross breeds (Table 1).

Table 1: The overall prevalence of mastitis using CMT

Breed	No. of animals examined	No. of positive (%)	χ^2 (p value)
Crossbred	166	61 (36.7)	13.48 (0.021)
Local Fogera	136	24 (17.6)	-
Total	302	85 (28.2)	-

Table 2: Prevalence of subclinical and clinical mastitis at cow level and quarter using CMT

Forms of mastitis	Breed	No. of cows examined	Positive (%)	χ^2 (p value)	No. of quarters examined	Positive (%)	χ^2 (p value)
Subclinical	Cross	166	53 (31.9)	-	602	107 (17.8)	1.66 (0.00)
	Local	136	23 (16.7)	14 (0.001)	508	27 (5.3)	-
	Total	302	76 (25.2)	-	1110	134 (12.3)	-
Clinical	Cross	166	8 (4.8)	-	-	-	-
	Local	136	1 (0.7)	-	-	-	-
	Total	302	9 (3.0)	-	-	-	-

Table 3: The prevalence of mastitis in breeds in relation to their lactation stages (days) and parity

Breeds	Risk factor (days)	No. of examined	No. of affected	Prevalence (%)	χ^2 (p value)	df
Cross	0-60	46	25	54.3		
	61-120	32	9	28.1		
	121-180	32	7	21.9	10.06 (p = 0.039)	4
	181-240	33	10	30.3		
	Above 240	23	10	43.5		
	Total	166	61	36.7		
Local	0-60 days	40	12	30.0		
	61-120	32	3	9.4		
	121-180	26	2	7.7	6.96 (p = 0.137)	4
	181-240	16	2	12.5		
	Above 240	22	5	22.7		
	Total	136	24	17.6		
Cross	1-2 birth	59	14	23.7		
	3-4 birth	71	23	32.4	18.74 (p = 0.00)	2
	≥5 birth	36	24	66.7		
	Total	166	61	36.7		
Local	1-2 birth	63	5	7.9		
	3-4 birth	49	8	16.3	17.26 (p = 0.00)	2
	≥5 birth	24	11	45.8		
	Total	136	24	17.6		

The result showed that the prevalence of clinical and subclinical mastitis as 3 and 25.2%, respectively (Table 2). Of 1208 quarters examined 23 (1.9%) were blind, leaving 1185 quarters functional. Subclinical and clinical prevalence at cow level was 53 (31.9%) and 8 (4.8%) in crossbreds and 23 (16.7%) and 1 (0.7%) in local Fogera breeds, respectively (Table 2) whereas the prevalence of mastitis on quarter bases was 12.3% (Table 2).

The results showed significantly higherinfection ($p<0.05$) in cows with early and late lactation stages than cows with mid lactation stage in crossbreds but it was insignificant ($p>0.05$) in local breeds (Table 3). There was a significant difference in the prevalence of mastitis in cows with different parity number ($p<0.05$). The prevalence of mastitis increased with an increase in parity number (Table 3).

The prevalence of mastitis was higher in cows with lesion on the udder or teat skin than in cows without this factor and in cows with poor hygiene than in good hygiene and the results showed significantly higher infection ($p<0.05$) (Table 4).

Milk sample of 139 quarter which is positive with CMT were cultured for microbiological examination and 71.2% (99) were yielded bacteria. The bacterial isolates and frequency of isolation were shown in Table 5. The predominant isolates from both types of mastitis were *Staphylococcus* species (72.2%) and followed by *Streptococcus* species (16.4%) of which *Streptococcus agalactiae* was accounted for 8.8%. *Corynebacterium bovis* and *Bacillus* species were the least frequently isolated (1.3% each). *Staphylococcus aureus* and *E. coli* were isolated from clinical and subclinical mastitis whereas the others were isolated only from subclinical mastitis (Table 5).

The study showed that the overall prevalence of mastitis in local Fogera and crossbreed cows in the study area to be 28.2% which is in agreement with the reports on bovine mastitis reported by Biffa *et al.* (2005) and Almaw *et al.* (2008). On the other hand, the present finding was lower than the reports of Tolossa (1987) (53.5%). The variability between reports could be ascribed to difference in management of the farms, breeds considered or agro-climatic conditions.

Clinical prevalence at cow level was 4.8% in crossbreds and 0.7% in local breeds. The clinical prevalence in crossbreds in this study was comparable with that of Almaw *et al.* (2008). However, the present finding was lower than that reported by Workineh *et al.* (2002), Biffa *et al.* (2005) and Lakew *et al.* (2009). The prevalence of subclinical mastitis in crossbreds at cow

Table 4: Mastitis prevalence in relation to hygiene and lesion on udder/or teat risk factors

	Hygiene			Lesion on udder/teats		
Cows	Poor	Good	χ^2 (P)	Lesion	No. of lesion	χ^2 (P)
Total number examined	98.0	204.0	44.78 (0.012)	31.0	271.0	72.16 (0.032)
Total number infected	64.0	21.0	-	28.0	57.0	-
Prevalence (%)	65.3	10.3	-	90.3	21.4	-

Table 5: Frequency of bacterial isolated from bovine clinical and subclinical mastitis

Microorganisms	Clinical frequency	Subclinical (frequency)	Total	Prevalence (%)
Staphylococcus aureus	4	12	16	20.3
CNS	-	41	41	51.9
Streptococcus agalactiae	-	7	7	8.8
Streptococcus uberis	-	2	2	2.5
Streptococcus dysgalactiae	-	4	4	5.1
Micrococcus species	-	3	3	3.8
Actinomyces pyogenes	-	2	2	2.5
Corynebacterium bovis	-	1	1	1.3
Bacillus species	-	1	1	1.3
Escherichi coli	2	0	2	2.5
Total	6	73	79	100.0

level was 31.9% which is in agreement with the reports of Almaw *et al.* (2008). However, Lakew *et al.* (2009) reported a higher prevalence (38%) in the Asella, South East Ethiopia compared to the present finding. The study revealed that cow's hygiene significantly affected the prevalence of subclinical mastitis ($p<0.05$) which was compatible with the research of Lakew *et al.* (2009).

The prevalence mastitis for cows gave birth 5 and above was 66.7% for crossbred cows whereas 45.8% was recorded in indigenous Fogera breed. This agrees with Rahman *et al.* (2009) who had reported prevalence of 12.3 and 65% during the first and last parity, respectively. The higher the parity number, the higher the prevalence. This was in agreement with the research of Biffa *et al.* (2005) and Tesfaye (1995) who had reported that there had been an increase in prevalence of subclinical mastitis with increasing number of parity significantly ($p<0.05$). In the present study, early and late lactation stages were also found to increase occurrence of mastitis significantly ($p<0.05$) in crossbred lactating cows but it was insignificant ($p>0.05$) in local Fogera lactating cows. This could be associated with the possibility of exposure to infectious agents with increased number of parity. According to Erskine (2001) primiparous cows have more effective defense mechanism than multiparous cows. The study showed that breed was significantly ($p<0.05$) affect the occurrence of mastitis.

This is inline with Lakew *et al.* (2009) who found significant difference between crosses and local Arsi breed and Biffa *et al.* (2005) found significant difference between local Zebu, Holstein-Frisian and Jersy breeds. Increased milk yield from genetic selection may be accompanied in genetic susceptibility to mastitis (Schutz, 1994). Therefore, the lower prevalence in local Fogera breeds in this study could be associated with difference in genetically controlled physical barrier like streak canal sphincter muscle, keratin in the teat canal or shape of teat end where pointed teat ends are prone to lesion (Seykora and McDaniel, 1985). In addition to physical barrier, difference in occurrence of mastitis in these breeds could arise from differences in cellular immunity (Erskine, 2001).

In this study, CNS were the predominant pathogens involved constituting 51.9% of all isolates. This finding is in agreement with the research done by Hussein (1999) and Almaw *et al.* (2008) in Ethiopia. Dego and Tareke (2003) and Workineh *et al.* (2002), reported isolation of CNS lower than the present study in southern region and Addis Ababa, Ethiopia. CNS are regarded as minor pathogens and normal inhabitants of bovine mammary gland and usually are mentioned in association with a slight increase in somatic cell count (Rainard and Poutrel, 1988). However recently, CNS were isolated from bovine and other dairy animals mastitic milk samples (Almaw *et al*., 2008; Lakew *et al*., 2009; Tesfaye *et al*., 2010).

The prevalence of mastitis in cows with udder/or teat lesion was higher (90.3%) than compared to those without udder/or teat lesion (21.8%) and out of the total isolates from cows with udder/or teat lesion, CNS took the higher proportion. Thus, the effect of udder/or teat lesion on the occurrence of mastitis in this study was significant ($p<0.05$). In a study conducted in Israel after experimental infection of quarters with CNS, it was possible to isolate CNS after months indicating CNS could result in chronic infection (Leitner *et al*., 2000).

S. aureus (20.3%) was the second isolate next to CNS and was closely comparable with the findings of

Almaw *et al.* (2008), Sumathi *et al.* (2008) and Lakew *et al.* (2009). However, the present finding was lower than that of Workineh *et al.* (2002) and Dego and Tareke (2003). The relatively high prevalence of *S. aureus* in this study could be associated with absence of dry cow therapy and post milking teat dipping, the invariably hand milking practice and low culling rate of chronically infected cows. *Streptococci* species were also among the dominant (16.4%) bacterial population as mastitis pathogens in the study area. *Str. agalactiae* (8.8%) and *Str. dysgalactiae* (5.1%) were the dominant species. *Str. uberis* (2.5%) was isolated at a lower rate. This finding was closely similar with the finding of Wtsadik (2009) and Hassen (2009) who reported isolation rates of 8.7% *Str. agalactiae* and 4% *Str. uberis*, respectively. This finding was also comparable with that of Dego and Tareke (2003) who reported isolation rates of 13.1% *Str. agalactiae*, 5.6% *Str. dysgalactiae* and 5.1% *Str. uberis*. Bishi (1998) reported higher isolation rate (27%) for *Str. agalactiae* and lower (0.5%) for *Str. dysgalactiae* compared to the current finding. Bishi's finding on *Str. uberis* (1.9%) was in agreement to the present finding (2.5%).

Actinomyces pyogenes was also isolated with a proportion of 2.5%. The present finding was in agreement with that of Hassen (2009) who reported 3.27%. This finding was lower than the finding of Bisrat (1999) and Oudessa (1997) with an isolation rate of 5.8 and 6.1%, respectively.

E. coli bacteria isolation (2.5%) in this study, agrees with Mengistu (1986) who reported 3.14 and 3.64%. But lower than that of Iqbal *et al.* (2004) and Sumathi *et al.* (2008) who reported 46.98 and 20%, respectively. Sumathi *et al.* (2008) reported higher incidence of *E. coli* mastitis may be due to poor hygienic conditions as *E. coli* originates from the cow's environment and infect the udder via the teat canal.

In this study, isolation of environmental pathogens *Str. uberis* and enterobacteriae especially coliforms were low. There is a common understanding that with increasing herd size, manure disposal and sanitation problems also increase which will lead to build up of bacterial population (coliforms and environmental streptococci) in the cow's immediate environment (Sumathi *et al.*, 2008). The number of hours dairy cows kept in door is also a factor that will increase the possibility of contact of teats with the environmental pathogens according to Sumathi *et al.* (2008).

CONCLUSION

This study revealed that host risk factors significantly influence the prevalence of mastitis. Subclinical mastitis was more important when compared to clinical mastitis. Crossbred cows were affected more than local fogera. The pathogens found involved were Coagulase Negative Staphylococci (CNS), *S. aureus*, *Str. agalactiae*, *Str. dysgalactiae*, *Str. uberis*, *Micrococcus* species, *C. bovis*, *A. pyogens*, *Bacillus* species and *E. coli*. Among these, the most frequent isolates were CNS (51.9% of the total isolates), *S. aureus* (20.3%), *Str. agalactiae* (8.8%) and *Str. dsgalactiae* (5.1%). This study showed that mastitis is a major health problem of dairy cows in the study area and undoubtedly will have an adverse effect on productivity of dairy industry and hence warrants serous attention.

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