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Bovine Mastitis: Prevalence, Risk Factors and Bacterial Isolation in Small-Holder Dairy Farms in Addis Ababa City, Ethiopia

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Abstract: A cross-sectional study was conducted on lactating dairy cows to determine the prevalence, risk factors and bacterial causative agents in smallholder dairy farms from October, 2008 to March, 2009. The result of this study revealed that the overall prevalence of mastitis was found to be 52.27% (15.41% clinical and 36.86% sub-clinical cases). Among the total (1324) quarters examined, 61 (4.61%) had blind teats. The prevalence of mastitis showed statistically significant difference between, number of parity, stage of lactation, body condition of animals, farm hygiene and udder washing ($P<0.05$). However, there was no statistically significant difference noted among the risk factors, herd size, age of the animals, previous mastitis and use of contaminated towel($P>0.05$). From CMT positive (146) samples sent to a laboratory for microbiological examination, 71 bacterial isolates were identified. The majority of isolates were *Staphylococcus aureus (S.aureus)* (21.13%) followed by *Streptococcus agalactiae (S.agalactiae)* (18.31%) and *Coagulase negative staphylococci (CNS)* (11.27%) and the lowest isolation rate was for *Micrococcus species* (2.82%). The Other species which isolated include *Actinomyces pyogenes(A.pyogenes), Klebsiella species* and *Streptococcus uberis(S.uberis)* (4.23% each), *Streptococcus dysgalaciae(S. dysgalaciae)* (5.63%), *Corynebacterium bovis(C.bovis)* and *Escherichia coli(E.coli)* (7.04% each), *Streptococcus pyogens(S.pyogens)* (8.45%) and *Bacillus species* (5.63%). This study demonstrated that both clinical and sub clinical mastitis is common in smallholder dairying in Addis Ababa city and that some of the risk and protective factors for mastitis can be addressed by practical management of dairy cows.

Key words: Epidemiology · Species · Husbandry · CMT Addis Ababa · Ethiopia

INTRODUCTION

Mastitis in dairy cows occurs worldwide and can be caused by infections with bacteria, yeast and fungi [1]. Bacteria are the most common cause of intramammary infection and the range of causal bacterial species varies with geographical location and management. In previous studies in developing countries, yeast or fungi represent a greater proportion of isolates than those from developed countries, possibly indicating different exposure levels, host susceptibility, or increased contamination of collected milk samples [2].

In Ethiopia, the available information indicated that bovine mastitis is one of the most frequently encountered diseases of dairy cows. According to Hussein *et al.* [3] of the major diseases of crossbred cows in Addis Ababa milk shed, clinical mastitis was the second most frequent disease next to reproductive diseases, in which 171 cows out of 556 were found to be affected. Generally, the prevalence of clinical and sub clinical mastitis in different parts of Ethiopia range from 1.2 to 21.5% and 19 to 46.6%, respectively [2-8]. Clinical (4.9%) and sub clinical (45.5%) mastitis was reported in Bahir Dar, Ethiopia [9]. In the same study area after ten years a prevalence of 40% sub clinical mastitis was reported [10]. These studies showed that bovine mastitis is among the major health problems hindering dairy productivity in Ethiopia, which requires the development of methodologies of control program under the prevailing husbandry system. However, according to Hussein *et al.*[3] so far efforts have been

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concentrated only on the treatment of clinical cases. On the other hand, losses from mastitis have been attributed mainly to decreased milk production from sub clinical mastitis [11].

Even though the disease has been known locally in Ethiopia, it has not been studied systematically, making information available on the prevalence of disease and associated with economic loss inadequate. The objectives of the present study were therefore to estimate the prevalence of mastitis, associated risk factors and to identify the major mastitis pathogens in cross breed dairy cows in Addis Ababa city smallholder dairy farms.

MATERIALS AND METHODS

Study Area: Addis Ababa is the capital city and administration center for the Federal Democratic Republic of Ethiopia. Currently there are 10 "*Kifle Ketemas*" in Addis Ababa city administration delineated on the basis of geographical set up, population density, asset and service providers' distribution and convenience for administration [12]. Addis Ababa is situated at latitude of 9°3'North and 38°43'East [13]. It lies in the central highlands of Ethiopia at an altitude of 2500 m.a.s.l. It has an average rainfall of 1800 mm per annum. The annual average maximum and minimum temperature is 26°C and 11°C, respectively; with an overall average of 18.7°C. Highest temperatures are reached in May. The main rainy season extends from June to September. Addis Ababa has a relative humidity varying from 70% to 80% during the rainy season and 40% to 50% during the dry season. The human population is estimated at about 3 million inhabitants [14].

Study Design: The study involved cross-sectional observation in a multistage sampling technique [15]. Four sub cities were selected from Addis Ababa city based on dairy cow population. For estimation of disease prevalence with an expected prevalence of 40% and precision level of 95%, a sample size of 369 milking cows was determined based on the formula described by Putt *et al.* [16]. From each sub city, nine farms were randomly selected followed by a random sampling of 10 lactating cows. However, due to the lack of cooperation in some farm owners, 331 lactating cows were used for this study.

Data Collection: A semi-structured questionnaire was developed and pre-tested and all information pertinent to the study objectives was recorded. Data collected include, age, parity and lactation stage. Udder and milk abnormalities (injuries, blindness, swelling, milk clots, abnormal secretion, etc.) and the management practices were recorded.

Preparing Udders and Teats: Each teat end was scrubbed vigorously with a pledged of cotton moistened (but not completely wet) with 70% ethyl alcohol. Recontamination of teats during scrubbing, was avoided by scrubbing, the teats on the far side of the udder first, then those on the near side. Separate pledged cotton was used for each teat.

Collection of Milk Samples: 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. After collection, the sample was placed in ice box and transported to the laboratory. The samples were either cultured or stored at 4°C until cultured within few days.

CMT Screening: The California Mastitis Test (CMT) was carried out according to the method described by Quinn *et al*. [17]. 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, in a horizontal plane for 15 seconds.

Bacterial Isolation: Bacteriology was performed on foremilk samples from those strong CMT reactive quarters for culture due to the shortage of resources. Identification of mastitis pathogens was carried out following microbiological procedures for diagnosis of bovine udder infection described in Quinn *et al*. [17]. 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. One standard loop (0.01ml) of milk sample was streaked on 7% blood agar. The inoculated plate was incubated aerobically at 37° C. The plates were checked for growth after 24, 48 and up to 72 hours to rule out slow growing microorganisms such as *Corynebacterium* species. For primary identification, colony size, shape, color, hemolytic characteristics, Grams reaction and catalase production were used.

For confirmation, biochemical tests were used after sub culturing isolated distinct colony on to a nutrient agar. Interpretation was made according to NMC [18]. The culture was considered negative if no growth occurs after 72 hours of incubation. Isolation of two or more colonies from a quarter sample was considered contaminated and the result was disregarded.

Data Analyses: 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. Age of the animals was determined from birth records and dentition characteristics and categorized as young adults (≤ 4 years), adults (>4 to ≤ 8 years) and old (>8 years). Stage of lactation was categorized as early (1–120 post partum), middle lactation (121-240 days) and late lactation (greater than 240 days). Parity was also categorized as few (<3 calves), moderate (3–5 calves) and many (>5 calves). The other risk factors were categorizes as yes or no for the presence or absence of the risk in the hypothesis, respectively. In case of prevalence of mastitis, factors were first screened by running univariate logistic regression between each risk factor and occurrence of mastitis then risk factor with a P-value less than 0.25 were further analyzed in multiple logistic regression. All the necessary statistical analysis was performed using Stata version 9.0 for windows (Stata Corp. College Station, TX).

RESULTS

Of the total 331 animals included for this particular study, the results showed that prevalence of mastitis was 52. 27% (clinical and sub-clinical mastitis 15.41% and 36.86 %, respectively). Blind teat was examined in 61 (4.61%) out of 1324 quarters. Univariable logistic regression showed that there was a statistically significant difference (P< 0.05) observed between the prevalence of mastitis and potential risk factors such as, age, parity, body condition, farm hygiene and udder cleaning. However, herd size, previous mastitis and use of contaminated towel were not statistically significant (P >0.05) (Table 1).

Further multivariable logistic regression analysis showed that parity, stage of lactation, body condition, farm hygiene and udder cleaning were statistically significant (P < 0.05). However, herd size, age, previous mastitis and use of towel were not statistically significant (P<0.05) (Table 2).

Bacteriological Examination: One hundred and forty six (146) quarter samples were used for bacterial culture from the total mastitis cases due to the resource limitation. Out of this, 71 (64.54%) samples were culture positive single colony, 19 (17.27%) showed mixed growth and 20 (18.18%) yield no bacteria. In this study, *S. aureus* were the most

Table 1: Univariate logistic regression of the risk factors for the prevalence of mastitis

Variables	Levels	No.	Positive	Proportion	OR (95%CI)	P-value
Herd size	<15	70	33	47.14	1.00	1.00
	≥15-20	134	68	50.74	1.16	0.63
	≥20	127	72	56.69	1.47	0.19
	<3	26	5	19.23	1.00	1.00
Parity	3-5	188	96	51.06	4.38	0.00
	>5	117	72	61.54	6.72	0.00
	1 to 3	91	42	46.15	1.00	1.00
Stage of lactation	3 to 4	64	32	50.00	1.17	0.64
	>4	176	99	56.25	1.50	0.12
Previous mastitis	Yes	157	82	52.23	1.00	1.00
	No.	174	91	52.30	1.01	0.99
Body condition	Poor	58	57	98.27	1.00	1.00
	Good	273	116	42.49	0.01	0.00
Age in years	≤4	68	23	33.82	1.00	1.00
	>4- ≤8	174	97	55.75	2.47	0.00
	>8	89	53	59.55	2.88	0.00
Farm hygiene	Poor	192	123	64.06	1.00	1.00
	Good	139	11	7.91	0.70	0.00
Udder washing	No	192	118	52.48	1.00	1.00
	Yes	139	11	51.94	.16	0.00
Use of towel	No	62	27	43.55	1.00	1.00
	Yes	269	146	54.28	1.54	0.13

Table 2: Multivariate logistic regression of risk factors for the occurrence of mastitis

Risk factors	Odds Ratio	Std. Err.	z	P>z
Herd size	1.40	0.30	1.54	0.12
Age	0.77	0.22	-0.93	0.35
Parity	2.06	0.69	2.16	0.03
Stage of lactation	1.56	0.28	2.48	0.01
Previous mastitis	0.77	0.28	-0.73	0.47
Body condition	0.07	0.04	-4.86	0.00
Farm hygiene	0.07	0.05	-4.12	0.00
Udder cleaning	0.16	0.10	-3.01	0.00
Use of towel	1.20	0.47	0.46	0.64

Table 3: Frequency distribution of bacterial isolates from strong CMT reactive quarters

No	Isolates	Frequency	Proportion
1	*S. aureus*	15	21.13
2	*A. pyogens*	3	4.23
3	*Klebsiella spp.*	3	4.23
4	*CNS**	8	11.27
5	*S. agalactiae*	13	18.31
6	*Micrococcus spp.*	2	2.82
7	*S. dysgalactiae*	4	5.63
8	*S. uberis*	3	4.23
9	*C. bovis*	5	7.04
10	*S. pyogens*	6	8.45
11	*B. spp.*	4	5.63
12	*E.coli*	5	7.04
	Total	71	100%

CNS Coagulase Negative Staphylococci*

predominant pathogens constituting (21.13%) of all isolates followed by *S. agalactiae* (18.31%) and CNS (11.27%) (Table 3).

DISCUSSION

In this study, clinical prevalence was 15.4% which is comparable with that of Sori *et al*. [19] and Karimuribo *et al*. [20] who reported that 16.11% and 14.2% prevalence in Ethiopia and Tanzania, respectively. However, the present finding was lower than that reported by Workineh *et al*. [7] (25.1%) in Addis Ababa, Ethiopia. Mastitis is a complex disease and the difference in results could be due to difference in management system between the farms. The prevalence of sub clinical mastitis based on CMT in the present study (36.86%) was comparable to the finding of Bishi [4] NMC and Karimuribo *et al*. [18, 20] who reported that 34.30%, 34.40% and 36.67% in Addis Ababa, Bahir Dar and in and around Sebeta, Ethiopia, respectively. However, Shirmeka [10] reported a higher prevalence (40%) in Bahir Dar compared to the present finding. This can most probably be attributed to variations in the distribution of mastitis risk factors reflecting differences in case definition, laboratory techniques, study design, climate, the level of management and animals studied. In this study, clinical mastitis was identified by the assistance of individual dairy farmer on the basis of clinical signs including abnormal milk, or a hard or swollen udder, or both. Mastitis, in both its clinical and sub clinical forms was found to be common in Addis Ababa smallholder dairy farms and contagious pathogens were found to be more common than environmental pathogens, with *S. aureus* being isolated most frequently.

In this study as well as in other similar studies, overwhelming cases of mastitis were sub clinical compared to clinical mastitis [3, 5, 8]. In Ethiopia, the sub clinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases [3] while the high economic loss could come from sub clinical mastitis. According to Radostits *et al*. [21], an infected quarter showed 30% and a cow 15% reduction in milk yield. Usually Ethiopian farmers especially smallholders are not well informed about the invisible loss from sub clinical mastitis [3] since dairying is mostly a sideline business among these farmers. This was also true in the study area that none of the dairy farm owners screened their cows for sub clinical mastitis except seeking veterinarian's assistance at times of clinical cases. To maximize milk production in the city, bureau of agriculture should introduce systems that increase awareness on sub clinical mastitis to dairy farm owners.

Among the risk factors considered, number of parity, stage of lactation, body condition, farm hygiene and udder cleaning were found to be statistically significant ($P<0.05$). Cows in parity number greater than three had significantly higher mastitis prevalence ($P < 0.05$) than those 2-3 and primiparous. According to Erskine [22] primiparous cows have more effective defense mechanism than multiparous cows. The prevalence of mastitis was found to be higher in animals with poor body condition compared to good body condition. This could probably associate with the ability of the immune system of an animal to defend infection causing agents. Udder washing was also found to be associated with the occurrence of mastitis, higher in animals/farms where udder washing is not practiced. However, herd size, age of the animals, previous mastitis and use of towel were not statistically significant ($P>0.05$).

Out of 1324 quarters examined, 61 (4.61%) were blocked indicating mastitis was a problem. The isolation rate of *S. aureus* (21.13%) in this study was higher than

the findings of [4; 17 who reported 9% and 10.69% prevalence in Addis Ababa, respectively. However, the present finding was lower than that of Workineh *et al.*

Kerro and Tareke [7, 8] where *S. aureus* accounted for 139.2% and 40.5% of the isolates, respectively. The relatively high prevalence of *S. aureus* in this study among the isolates could be associated with total absence of dry cow therapy and post milking teat dipping, the invariably hand milking practice, low culling rate of chronically infected cows (culling was usually due to feed shortage, aging and reproductive problem) and limited knowledge of farmers on segregation as a control option. The primary reservoir of contagious pathogens including *S. aureus* is infected quarter and the exposure of uninfected quarter is limited to the milking process [23].

Streptococci species were also among the dominant (16.3%) bacterial population as mastitis pathogens in this study. *S. agalactiae* (18.31%) and *S. dysgalactiae* (5.63%), *S. Uberis* (4.23%) were the dominant species. *Micrococcus spp. (*2.82%) was isolated at a lower rate. This finding was comparable with that of Kerro and Tareke [8] who reported isolation rates of 13.1 % *S. agalactiae*, 5.6% *S. dysgalactiae* and 4.23% *S. uberis*. Almaw [24] reported *S. agalactiae* (8.15%) and *S. dysgalactiae* (6.67%) were the dominant species and *S. uberis* (1.48%) was isolated at a lower rate. Bishi [4] reported higher isolation rate (27%) for *S. agalactiae* and lower (0.5%) for *S. dysgalactiae* compared to the current finding. The explanation given for *S. aureus* could also be a factor for *S. agalactiae* and *S. dysgalactiae* relative high isolation rate since both of them are contagious pathogens.

The present study showed that contagious mastitis pathogens were the predominant isolates that might be due to lack of effective udder washing and drying, post milking teat dip and drying, inter-cow hand washing and disinfection in the milking routine of the area. Contamination of milkers' hands, wash clothes and milking machine cups by milk from infected quarter has been reported to quickly lead to spread of mastitis.

In this study, *S. aures* was the predominant pathogens involved constituting (21.13%) of all isolates. The low level isolation of CNS (11.27%) in this study disagrees with the findings of Bishi and Putt *et al*.and Quinn *et al.* [4, 16, 17] in Ethiopia who reported 54%, 42% and 49.63% respectively. This might be due the number of samples used for these studies. The isolation rate of CNS was 2.5% in southern region, Ethiopia [8] which is lower than the present prevalence study.

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REFERENCES

1. Bennedsguard, T.W, C. Enovoldsen, S.M. Thamsborg and M. Varsts, 2003. Effect of mastitis treatment and Somatic cell counts on milk yield in Danish organic Dairy cows. J. Diary Sci., 86: 3174-3183.
2. Lemma, M., T. Kassa and A. Tegegene, 2001. Clinically manifested major health problems of crossbred dairy herds in urban and periurban production systems in the central high lands of Ethiopia. Trop. Anim. Hlth. Prod., 33: 85-89.
3. Hussein, N., T. Yehualashet and G. Tilahun, 1997. Prevalence of mastitis in different local and exotic breeds of milking cows. Eth. J. Agr. Sci., 16: 53-60.
4. Bishi, A.S., 1998. Cross-sectional and longitudinal prospective study of bovine clinical and sub clinical mastitis in periurban and urban dairy production systems in the Addis Ababa region, Ethiopia. Faculty of Veterinary Medicine, Addis Ababa University School of Graduate Studies and Freie Universitat, Berlin, Berlin, MSc Thesis.
5. Kassa, T., G. Wirtu and A. Tegegne, 1999. Survey of mastitis in dairy herds in the Ethiopian central highlands. Ethio. J. Sci., 22: 291-301.
6. Mungube, E.O., 2001. Management and economics of dairy cow mastitis in the urban and periurban areas of Addis Ababa (Addis Ababa milk shed). Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, MSc Thesis.
7. Workineh, S., M. Bayleyegne, H. Mekonnen and L.N.D. Potgieter, 2002. Prevalence and aetiology of mastitis in cow from two major Ethiopian dairies. Trop. Anim. Hlth. Prod., 34: 19-25.
8. Kerro, O. and F. Tareke, 2003. Bovine Mastitis in Selected Areas of Southern Ethiopia. Trop Anim Hlth Prod., 35: 197-205.
9. Mekuria, M., 1986. Prevalence and aetiology of bovine mastitis in Bahir Dar. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, DVM Thesis.

10. Shirmeka, G., 1996. Prevalence and Etiology of Sub clinical Mastitis in Friesian Indigenous Zebu Crosses and Indigenous Zebu Breeds of Dairy Cows in and around Bahir Dar. Debre Zeit: Addis Ababa University, Faculty of Veterinary Medicine, DVM Thesis.
11. Degraves, J. and J. Fetrow, 1993. Economics of mastitis and mastitis control. In: Hunt, E. (ed.), The Veterinary Clinics of North America, Food Animal Practice, Update On Bovine Mastitis. USA: W.B. Saunders Company, pp: 421-430.
12. AACA (Addis Ababa City Administration), 2004. Proclamation for establishment of "Kifle Ketema" and "Kebele" in Addis Ababa city, Addis Ababa, Ethiopia.
13. ILCA (International Livestock Centre for Africa), 1994. Annual Program Report, Addis Ababa, Ethiopia.
14. NMSA (National Meteorological Service Agency), 1999. Rainfall and Temperature data, Addis Ababa, Ethiopia.
15. Thrusfeild, M., 1995. Veterinary Epidemiology. 2nd Ed. Blackwell: Oxford, UK.
16. Putt, S.N.H., A.P.M. Shaw, A.J. Woods, L. Tyler and A.D. James, 1987. Veterinary epidemiology and economics in Africa. A manual for use in the design and appraisal of livestock health policy. ILCA Manual No. 3. 2nd Ed. International Livestock Centre for Africa: Addis Abeba, Ethiopia, pp: 35.
17. Quinn, P.J., M.E. Carter, B.K. Marke and G.R. Carter, 1999. Clinical Veterinary Microbiology. London: Mosby International Limited.
18. N.M.C., 1990. Microbiological procedures for the diagnosis of bovine udder infection, 3 rd ed. Arlington VA: National Mastitis Council Inc.
19. Sori, H., A. Zerihun and S. Abdicho, 2005. Dairy Cattle Mastitis In and Around Sebeta, Ethiopia. Intern J. Appl. Res. Vet. Med., 3(4): 332-338.
20. Karimuribo, E.D., J.L. Fitzpatrick, C.E. Bell, E.S. Swai, D.M. Kambarage, N.H. Ogden, M.J. Bryant and N.P. French 2006. Clinical and sub clinical mastitis in smallholder dairy farms in Tanzania: Risk, intervention and knowledge transfer. Preventive Veterinary Medicine, 74: 84-8.
21. Radostits, O.R., D.C. Blood and D.C. Bay, 1994. Veterinary Medicine, A Text Book of the Diseases of Cattle, Sheep, Goats and Horses. 8th ed. London: Bailler Tindall, pp: 563-614.
22. Erskine, R.J., 2001. Mastitis control in dairy herds. In: Radostits, O.M. (ed.): Herd Health: Food Animal Production. 3rd ed. Philadelphia: W. B. Saunders Company, pp: 397-435.
23. Fox, K.F. and M.J. Gay, 1993. Contagious mastitis. In: Hunt, E. (ed.) and the Veterinary Clinics of North America, Food Animal Practice and Update on Bovine Mastitis. USA: W.B. Saunders Company, pp: 475-488.
24. Almaw, G., 2004. A cross-sectional study of bovine mastitis in and around Bahir dar and antibiotic resistance patterns of major pathogens. Addis Ababa University, Faculty of Veterinary Medicine, DVM Thesis.