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Full Length Research Paper

Mastits: Prevalence, risk factors and antimicrobial sensitivity patterns of bacterial isolates in dairy cattle at Holeta farm in Ethiopia

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The study was conducted at Holeta Agriculture research center over October 2011 to May 2012 to estimate prevalence of mastitis, assess risk factors, isolate bacterial causes and measure antimicrobial sensitivity patterns of isolates in dairy cattle. A total of 272 comprising 130 cross breed (Holstein x Boran), 75 Jersey and 67 Holstein Frisian were examined using California Mastitis Test (CMT) and clinical examination. An overall prevalence of 55.9% where sub- clinical and clinical mastitis accounted 52.4 and 3.3%, were respectively observed. Quarter level prevalence sub-clinical (28.3%) and clinical (1.2%) were observed. The blind teat accounted 6.0%. Major isolates were *Staphylococcus aureus* (39.5%), *Streptococcus species* (29.6%), *Escherichia coli* (19.7%), *Klebsiella pneumonia* (13.2%), *Enterobacter aerogen* (9.9%) and *Pseudomonas aeruginosa* (5.3%) in decreasing order. Animal factors such as age, breed and parity number have significant effect on prevalence of mastitis. Risk factors analysis revealed that prevalence was significantly differed with the age and breed. Thus, prevalence was relatively higher in adult cows (OR = 0.41), plurimiparas (OR = 2.6) and exotic breed (OR = 0.6) than those corresponding animals. Isolates were found highly sensitive to gentamycin while it was moderate to low for penicillin and amoxicillin. Based on the finding, effort should be made to control mastitis so as to ensure quality of milk, prevent economic loss and public health hazard.

Key words: Antimicrobial sensitivity, Holeta, mastitis, prevalence.

INTRODUCTION

Livestock play crucial role in the live hoods of the majority of Africans (Banai, 2002). Like in many developing countries, domestic animals play a crucial role in Ethiopia as source for traction power, income, in provision of milk and meat (Mekonnen et al., 1989). Ethiopia is known for its high livestock population, being the first in Africa and tenth in the world; the recent livestock population estimate amounts about 44.3 million herds of cattle, 23.6 million sheep, 23.3 million goats (CSA, 2004). In Ethiopia over 42% total cattle heads are milking cow which might represents the largest population of cattle production of the country. However, in comparison to other countries in Africa, Ethiopians consume less dairy products. The per capital consumption of milk in Ethiopia is as low as 17 kg per head while the average figure for Africa is 26 kg per head (Gebreweld et al., 2000).

Milk produced from cows provides an important dietary source for the majority of rural as well as a considerable

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number of the urban and peri-urban population. It is also one of the most important foods of humans and universally recognized as a complete diet due to its essential component (Javaid et al., 2009). However, in Ethiopia milk production often does not satisfy the country's requirements due to a multitude of factors; mastitis is among the various factors contributing to reduce milk production (Gebreweld et al., 2000). Mastitis is one of the most complex diseases of dairy cows that generally involve interplay between management practices and infectious agents, having different causes, degrees of intensity, and variations in duration and residual effects. Moreover, mastitis is the most important and expensive disease of dairy production. It results in severe economic losses from reduced milk production, treatment cost, labor cost, milk withheld following treatment and premature culling. Globally, the losses due to mastitis amount to about 53 billion dollars annually. Mastitis in both clinical and subclinical form is a frustrating, costly and extremely complex disease that results in a marked reduction in quality and quantity of milk (Harmon, 1994). Subclinical mastitis is a major problem affecting dairy animals all over the world. It causes enormous losses for breeders and consequently influences the national income of the country (Miller et al., 1993). Therefore, the objectives of this study were to determine the prevalence and isolate and identify the major causes, the associate risk factors as well as the antibiotic sensitivity patterns of the major isolates in the study area.

MATERIALS AND METHODS

Study area

The study was conducted at Holeta Research Center over October, 2011 to May, 2012. The research center comprises two dairy farms found at Wolmera and Ada Berga districts. Holeta is 28 km West of Addis Ababa, at 09°02'N latitude and 38°34' E longitudinal and latitude ranges 2060 to 3380 m.a.s.l. with high altitude (41%), mid altitude (59%) climatic zone having average temperature of 21°C and 900 to 1100 mm annual rainfall with bimodal pattern as short rain season from March-April and long rain season from July-October. Ada Berga is also at 64 km West of Addis Ababa and at altitude of 2435 m.a.s.l, average annual temperature of 17.5°C and rainfall 1143 mm.

Study population

Study animals were pure Holstein, Jersey and cross breed (Holstein x Boran) lactating dairy cows found in the center (Dairy cows are managed under small scale and semi- intensive management system often provided with some supplementary diet in addition to the natural and agricultural by-products.

Animals and farm management

All lactating (N=273) dairy cattle in the center were included in the study and among this Holstein, Jersey and Cross breed breeds (Holstein x Boran) accounts 67 (24.5%) cows, 75 (27.5) cows and 130 (56.5%) cows.

Methodology

A cross-sectional study was performed using questioner survey, clinical examination and laboratory diagnosis. The structured questioner includes age, breed, parity number, lactation stage and management dairy cattle as variables. The questioner was presented to milker's found in the center and farm.

Laboratory diagnosis

Detection of mastitis

Mastitis was detected using the California Mastitis Test (CMT) and clinical inspection of the udder for sub-clinical and clinical form of mastitis. Clinical inspection and other parameters were done according to Quinn et al. (2002). Information related to the previous health history of the mammary quarters and cause of blindness is obtained from case record sheets. Physical appearance of milk from each quarter was examined for the presence of clots, flakes and blood and viscosity.

CMT conducted according to Quinn et al. (2002) procedures and the outcomes ware graded as 0 and trace for negative while +1.2 and +2 and +3 for positive.

Sample collection and handing

The surface of udder and teats were brushing, cleaned and dried with clean towel. The teats were disinfected with cotton, soaked in 70% alcohol before milk sample collection. After discarding the first 3 milking stream, about 10 ml of milk sample was collected in to a sterile test tube and transporting to Holeta research Center laboratory using an ice box. The samples were stored at 4°C until examination.

Bacteriological isolation and characterization

Bacteriological examination of the milk was carried out for CMT positive results following standard procedures set by Quinn et al. (2002). The milk sample was kept at room temperature (25°C) for 15 min and vortex before plating on the standard bacteriological media (on MacConkey agar and blood agar.). Primary and secondary tests were performed for the purpose of identifying bacteria.

Antimicrobial sensitivity test

Antimicrobial susceptibility on bacterial isolates (8isolates) was performed using agar disc diffusion technique methods described by National Committee for Clinical Laboratory Standards (NCCLS, 1997). Four different types of Amoxacillin (25 µg), Gentamicin (10 µg), Spectnomycin (10 µg) and Penicillin G (10 units) identified isolated colony of bacteria was inoculated in BHI followed by overnight incubation at 37°C. The overnight culture was then swabbed on the surface of blood-Mueller Hinton agar followed by the application of antibiotic discs.

The effectiveness of a drug was determined by measuring the diameter of the zone of inhibition around the disc, the larger the diameter the more effective the drug. A scale of 0 to 3 was used to score the relative efficacy of various drugs against a given pathogen as presented (Holt et al., 1993). A zone diameter of 0 to 8

Table 1. Mastitis prevalence based on clinical examination and CMT result in dairy cattle at Holeta, Ethiopia.

Mastitis	Number of quarter	Percent (%)
Clinical mastitis	13	1.20
Sub-clinical mastitis	306	28.3
Blind	65	6.0
Apparently healthy	698	64.5
Total	1082	100

Table 2. Association between intrinsic animal factors and prevalence of mastitis, in dairy cattle at Holeta, Ethiopia.

Animal factors	No. of animals	No. positive	Prevalence (%)	χ^2 calculated	P-value
Age					
Young	161	76	47.2		
Adult	77	51	39.3	12.6	0.002
Old	34	25	8.5		
Total	272	152	55.9		
Breed					
Cross	130	81	62.3		
Jersey	75	44	58.7	9.0	0.011
Holstein	67	27	40.3		
Total	272	152	55.9		
Parity number					
1-4	212	109	51.4		
5-9	47	34	15.98	21.6	0.027
≥9	13	9	1.17		
Total	272	152	55.9		
Lactation stage					
1-120 days	98	49	48.02		
121-240 days	104	62	59.6	2.16	0.338
≥240 days	70	41	58.6		
Total	272	152	55.9		

mm scored 0 or 'R' for resistant, 9 to 15 scored + or slightly sensitive, 16 to 22 mm scored ++ or sensitive; 23 and above scored +++ or very sensitive (Quinn et al., 2002).

Data management and analysis

The data were entered and managed in MS Excel. SPSS version 16 statistical software's were applied for the data analysis. Both descriptive and analytical statistical techniques were used for data analysis. The differences in parameters like breed, management, lactation stage and parity on reproductive problems were analyzed by using χ^2 (Chi-square) technique and odd ratio is used to estimate the risk.

RESULTS

Two hundred seventy seven (N = 273) dairy cattle were incorporated in this study from Holeta research center. Physical examination and CMT result reveled, 28.3 and 1.2% udder quarter prevalence of sub-clinical and clinical mastitis respectively. 6.0% of udder quarter found to be blind (Table 1).

Animal factors such as age, breed and parity number have significant effect (P=0 .002, P=0.011) and P=0.027 respectively) on prevalence of mastitis. According to this study the stage lactation has no significant effect (P=0 .338) on prevalence of mastitis in dairy cattle (Table 2)

The prevalence of mastitis based on CMT or culture accounts young animals (73.5%), Cross breed (62.3%), Pluriparas (62.8%) and late lactation (56%) which are higher than adult (53.4%), exotic (50%), primiparas (39.5%) and early lactation (50%). The odd ratio for young/adult showed that the odds of prevalence of

Table 3. Determination of risk factors based on Odds Ratio (OR), in dairy cattle at Holeta, Ethiopia.

Animal factors	No. positive	No. negative	Total	Odd ratio (OR)
Age				
young	25 (73.5%)	9	34	
Adult	127(53.4%)	111	238	0.41
Total	152(55.9%)	120	272	
Breed				
Cross	81(62.3%)	49	130	
Exotic	71(50%)	71	142	0.6
Total	152(55.9%)	120	272	
Parity number				
Primparas	32 (39.5%)	49	81	
Pluriparas	120 (62.8%)	71	191	2.6
Total	152(55.9%))	120	272	
Lactation stage				
Early	49(50%)	49	98	
Late	103(56%)	71	184	0.69
Total	152(55.9%)	120	272	

Table 4. Bacteria isolated from dairy cows with subclinical mastitis**,** in dairy cattle at Holeta, Ethiopia.

S/N.	Types bacteria isolated	No. of bacterial isolate	Prevalence (%)
1	*Staphylococcus aurous*	60	39.5
2	*Streptococcus species*	45	29.6
3	*Pseudomonas aeroginosa*	8	5.3
4	*Escherichia coli*	30	19.7
5	*Klebsela pneumonia*	20	13.2
6	*Entrobacter aeroginosa*	15	9.9

mastitis are 0.41 times greater for adult than old dairy cattle. Table 3 indicates the odds of prevalence for Cross breed/Exotic, Primiparas/pluriparas, and early/late lactation.

From 152 culture positive samples, a total of six (6)bacterial species were isolated, the most prevalent being was *S. aurous* 39.5% (60/152). Other bacterial isolates included *Streptococcus* species 29.6% (45/152), *Escherichia coli* 19.7 (30/152), *Psudomanas aeroginosa* 5.3% (8/152), *Klebsiella pneumonia* 13.2% (20/152) and *Enterobater aerogen* 9.9% (15/152) (Table 4).

The study showed that sensitivity to antimicrobial agents was highest for gentamicin while it was moderate to low for penicillin and amoxicillin. On the contrary, the whole isolates were completely resistant to spectenomycin (Table 5).

DISCUSSION

A total of 272 milking cows (130 cross bred 75 Jersey and 67 Holstein Frisian) were examined using California Mastitis Test (CMT) and clinical examination. The prevalence of sub-clinical and clinical mastitis in the current study is lower than the study done by Lemma et al. (2001) who indicated 64.5% were positive for mastitis at cows' level among these, 26.3 and 38.1% were clinical and subclinical mastitis, respectively. Furthermore, the current study was also reviled lower prevalence as compared with the findings by Mekibib et al. (2009) at Holota area who indicated that the cow level overall prevalence of 71.0% of which 22.4% clinical and 48.6% of sub-clinical mastitis. They also indicated that the quarter level prevalence of mastitis accounted 44.9%.

The results of the present study are higher than previous findings of other researches done in different regions of the country such as 34.9% in southern Ethiopia by Biffa et al. (2005), 40% in South Ethiopia by Kerro and Tareke (2003), 44.1% by Girma (2010) and Hussein et al. (1997), 5.3% clinical and 19% sub-clinical mastitis at central Ethiopia and Abdelrahim et al. (1990) who found a

Table 5. Antimicrobial sensitivity patterns of major bacterial isolates from mastitic case in the center, in dairy cattle at Holeta, Ethiopia.

	Species of identified bacteria							
Anti-microbial agents	***E. coli***	***S. aurous***	***P. aeroginosa***	***K. pneumonia***	***S. uberis***	***S. dysagglacticia***	***E. aerogenosa***	***S. agglacticia***
Spectinomycin								
Zone of inhibition (mm)	-	-	-	-	-	-	-	-
Sensitivity patterns	R	R	R	R	R	R	R	R
Gentamicin								
Zone of inhibition (mm)	25	27	21	23	27	27	21	27
Sensitivity patterns	VS	VS	S	VS	VS	VS	S	VS
Penicillin-*G*								
Zone of inhibition (mm)	13	12	9	15	15	17	2	17
Sensitivity patterns	SS	SS	R	SS	SS	SS	S	S
Amoxicillin								
Zone of inhibition(mm)	12	15	-	17	20	18	19	18
Sensitivity pattern	SS	SS	R	S	S	S	S	S

prevalence of 45.8% in Sudan. On the contrary, this study showed that lower prevalence than previous findings done somewhere else by Kivaria et al. (2004) in Tanzania who reported a prevalence of 90.3%.

The current finding was in close agreement with study done by Benta and Habtamu (2011) who indicated an overall prevalence of 56.5% where the clinical and sub-clinical mastitis accounts 5.3 and 46.6% respectively. However, the quarter prevalence 31.4% of mastitis and 10.4% blind teats was higher than the current findingsThe difference in prevalence of mastitis in this finding may be due to the good management in the farm, difference in breed, sample size and the productivity of the dairy cattle.

The study showed that the major bacterial isolate at the Holeta research center was *S. aureus* (39.5%, *Streptococcus* species (29.6%), and *E. coli* (19.7%) as has been reported in other studies (Gitau et al., 2011; Haile, 2004). The other moderately prevalent isolates were *Klebsiella* spp., Enterobacter aerogenes and *Pseudomonas* spp. The study carried out in Zanzibar showed that *Staphylococcus* and *Streptococcus*spp were the most prevalent in the smallholder dairy farms (Gitau et al., 2011). *Streptoccoccus* species identified in this study is in close agreement with findings of Zerihun (1997) who reported 27% prevalence. However, the current prevalence is higher than 5.5% shown by Mekibib et al. (2009). On the contrary, the prevalence of *E. coli* and *Klebsiella* species are higher than the finding of Workeneh et al. (2002)

The current finding showed that most of the isolates were resistance to penicillin-G and amoxicillin. Similarly, a research done in India by Rajeev et al. (2010) indicated higher rate antibiotics resistance patterns for, penicillin G, amoxicillin and kanamycin. In accordance with this finding, higher sensitivity for gentamicin has also been reported by Goswami et al. (2002) and Langoni et al. (2000).

Abera et al. (2010) indicated that *S. aureus* which was the major cause of mastitis in this study too was found to be most susceptible to gentamicin and resistance to penicillin which is in close agreement with the current findings. A similar finding has also been reported by Mekonnen et al. (1989).

In general, antibiotic sensitivity test is widely used clinical investigation being followed worldwide in cases of bovine mastitis with the sole purpose to select most appropriate antimicrobial agent for therapeutic use. However, it is difficult to judge the clinical efficacy of an antimicrobial agent solely on the basis of *in vitro* test, as there are large variations in response among herds and within herds, due to type of organism involved, location of infected sites, degree of udder indurations, physico-chemical properties and kinetic behavior of antibiotics in udder and milk, site of injection and sensitivity of udder pathogens, lipid solubility and tissue protein binding of the drug, pH of milk and inflammatory exudates/ barrier at the site (Srivastava, 2000).

Conclusion

The current study revealed that there was high prevalence of mastitis and association of different intrinsic and extrinsic factors with the occurrence of mastitis. Furthermore, *S. aureus, Streptococcus* species, and *E. coli* still remain themost important causes of bovine mastitis in the study area. The major isolates in this study was found to be highly susceptible to gentamicin while it was moderate to low for penicillin and amoxicillin. Hence, hygienic milk production, early screening and treating mastitis using effective drugs are important in order to prevent and control mastitis occurring in the farm.

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