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Research Article

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Prevalence of bovine mastitis and identification of causative agents and predisposing risk factors in selected districts of Wolaita zone, Southern Ethiopia.

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Abstract

A cross-sectional study was conducted from December 2018 to April 2018 on lactating dairy cows todetermine the overall prevalence of bovine mastitis, identify associated risk factors and isolate thepredominant bacterial agents involved in causing mastitis in Damot gale and Damot sore districts. A total of 384local and cross bred milking cows were examined to determine theoverall prevalence of mastitis, to identify risk factor and to isolate and identify bacterial pathogens totest in study areas by using pre-testedquestionnaires, California mastitis test (CMT), microbial isolation and identification by using media culture and chemical tests. The overall mastitis prevalence was 23.7%, from which 5.7% were clinical and18%were sub-clinical mastitis. The major isolates of subclinicalmastitis cases were *Staphylococcus aureus* (44.3%), *Staphylococcus agalactiae*(19.7%),*Streptococcus dysgalactiae*(13.1%)and *Escherichia coli* (22.9%).Among the risk factors manure darainage system and use of bedding material haveshown statistically significant ($p<0.05$) difference in the prevalence of mastitis.Generally, the studyshowed that mastitis is an important problem which needs great attention in the study area.Therefore, appropriate control measures targeting the specific causative agents should be in place toreduce the impact of the disease. The farmers should also be aware of the impact of the disease andpractice hygienic milking, culling of chronic mastitis carriers and treating of clinically infected cows.

Keywords: Bacteria, Isolates, Mastitis, Prevalence, Risk Factors.

Introduction

Ethiopia has high livestock population with a total contribution of over 20% of Gross Domestic product and 41% of the Agricultural output (DACA, 2006). Wolaita zone also high number of livestock population; Current estimation shows that there are about 1,366,350 cattle, 330,348 sheep, 308,631 goats, 17,028 equine species and over 1,484,264 poultry (WZLFRD).

Livestock are the main economic source of the livelihood of the majority of human population by giving draft power supply for crop production and

transport as a source of meat, milk and egg. Livestock production also contributes to rural livelihoods, employment and poverty relief, integrating with and complementing crop production, acting as saving bank and providing buffer against risks (Sen and chander, 2003; Upton 2004).

Despite the all-around advantages of livestock at farmers and national level, productivity has remained very low. The main constraints are lack of sufficient and standard nutrition, poor husbandry practices, livestock diseases and lack of awareness. Poor livestock health services remain one of the main constraints to the development of livestock production in many developing countries. In sub-Saharan Africa, losses resulting from disease are estimated two billion dollars per year, of which half is attributable to direct losses from mortality and the other half to indirect losses as a consequence of reduced growth, fertility and ability to work (De Haan&Bekure 1991). Disease of various origins could be mentioned as among the major constraints of livestock production and productivity in various parts of the world. In Ethiopia the aggregate annual economic losses from animal diseases through direct mortality and reduced productive and reproductive performance were estimated at 150 million USD (Berhanu, 2002).

Livestock health problems were raised as the most important issue, constraining the livestock sector development of Wolaita zone in general. Therefore, alleviation of the impact and reducing the losses due to increased livestock diseases and animal health delivery system problems in different areas of Wolaita zone is the priority area to attain appropriate use of this huge stock and it needs to research in participatory and compulsory manner with farmers such that appropriate prevention and control methods of diseases will be designed, verified and implemented specially on mastitis.

In general, thorough information regarding the occurrence and distribution of major causative agents of the disease in Ethiopia in general and in Wolaita zone rural areas and its surrounding in particular is inadequate. Moreover, not enough investigations have been carried out to ascertain the effects of different risk factors on the prevalence of the disease. Taking these into consideration this study setted out the following objectives.

- To determine the prevalence of clinical and subclinical mastitis in dairy cattle in different management system and to identify major risk factors predisposing the disease in the area,
- To identify major pathogenic bacteria that cause mastitis as well as to investigate the effects of environmental and host risk factors on the prevalence of bovine mastitis.

Materials and Methods

Study area

The study was carried out in four selected woredas of Wolaita zone which are namely; Damot Gale, Boloso sore and Damot Sore. Wolaita zone is located in southern nations, nationalities and peoples' regional state at 390 km away from Addis Ababa. Geographically, Wolaita zone is located at 6.4^0-8.2^0 N latitude and 37.4^0 - 38.2^0E longitudes. The total land area covers 438,370 hectares, out of which 51.7% is cultivated land, 6.4% is cultivable and 11.9% is grazing land and 30% is others. Hilly, flat, steep slopes and gorges and a number of streams and mountains (WZFEDD and CSA, 2003) mark the area. The mean maximum temperature is 26.2°C and the average monthly minimum temperature is 11.4°C.Current estimation shows that there are about 1,366,350 cattle, 330,348 sheep, 308,631 goats, 17,028 equine species and over 1,484,264 poultry (WZLFRD,2011). With regard to the altitudes of the three districts, Damot gale is a high land area with an altitude of about 2700 meters abovesea level, Damot sore is mid land area that has got an altitude of 1300-2200 meters above sea level and Boloso sore is also midland area with an altitude of 1800 m.a.s.l (WZLFRD, 2011).

Study population and Sample Size Determination

The study was conducted on 384 lactating cows (local, Holstein-Friesian, Jersey and cross breeds) from study area. The samples were randomly selected based on the availability of lactating cows andbased on the owners' willingness. Simple random sampling method was applied for the selection of individual animals (lactating cows) in the district level. The sample size was determined by the formula given by Thrusfield (2007) considering an expected prevalence of 50% (Mekibib*et al*., 2009), 95% confidence level and 5% desired precision.

Study Design

A Cross sectional study was conducted. The study areas were selected purposely to handle mastitis occurrence assessment and associated risk factors. The lactating cows and quarter's level examination based on clinical manifestations for clinical mastitis and indirect test that was California Mastitis Test (CMT) for sub clinical mastitis and bacteriological examination (culture, stain, and biochemical tests on the pure isolates) were used to isolate the pathogens.

Simple random sampling technique was followed to select the study animal and the study was carried out from December 2018to April 2018 by collection of events associated with mastitis in lactating cows from 384dairy cows from selected kebeles in the study area.

Study Methodology

Clinical inspection of udder

The udder was first examined visually and then by palpation to detect possible fibrosis, inflammatory swellings, visible injury, tick infestation, atrophy of the tissue and swellings of supra mammary lymph nodes. The teat condition (color changes, swelling at or near the teat base, swelling or firmness at or near the teat end, openness of the teat orifice, teat skin condition, signs of vascular damage like petechial hemorrhage, etc.) was evaluated during clinical examination (More, 1989). Upon palpation, one can feel hot, painful swelling on udder and ventral abdomen and was manifested by loss of appetite, depression, recumbence and blood mixed milk in acuteclinical mastitis. In chronic mastitis, continuous or intermittent discharge of pus, clots, flakes or watery secretion will be seen from the udder (Chauhan and Agarwal, 2006).

Milk Samples Collection, Transportation and Storage

The milk samples were taken from cows not treated with either intra mammary or systemic antimicrobial agents. For good collection of milk sample the teat was wiped thoroughly with 75% ethyl alcohol. Approximately 10 ml of milk collected in to a sterile test tube after discarding the first three milking stream. Then the milk sample was held in ice box for transportation to the laboratory. In laboratory the sample was cultured immediately and some were stored at 40°C for a maximum of 24 hours until inoculated on a standard bacteriological media. After collection of the CMT positive milk sample from different quarters, all CMT positive samples were clearly labeled with the cows identification number using permanent marker on the test tube. And then transported with the icebox to the laboratory and processed immediately (Quinn *et al*., 2002). The isolation and identification of pathogenic bacteria performed at Wolaita Sodo Regional Laboratory.

California mastitis test (CMT)

The California mastitis test (CMT) was used as a screening test for sub-clinical mastitis. It was carried out according to the procedure described by Quinn *et al*. 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 CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 s. The CMT results were scored as 0 (negative), trace, 1(weak

positive), 2 (distinct positive) and 3 (strong positive) based on gel formation. All CMT scores of 0 and trace were considered as negative while CMT scores of 1, 2, and 3 were considered indicators of sub clinical mastitis. Positive cows were defined as having at least one quarter with CMT score of 1+ andmilk samples that formed coagulation or gel were taken for bacteriologicalidentification (Mellenberger and Carol, 2000).

Table 1: Interpretations for CMT

CMT score	Interpretation	Visible reaction
0	Negative	Milk fluid is normal
1	Trace	Slight precipitation
2	Weak positive	Distinct precipitation but not gel formation
3	Distinct positive	Mixture thickness with gel formation
4	Strong positive	Strong gel that is cohesive with a convex surface

Bacteriological identification

Milk sample, which were distinctly and strongly positive for CMT test, were used for bacteriological identification. Then,loopful of each milk sample was inoculated on blood agar base enriched with 5 % defibrinatedsheep blood (Wasilauskas*et al*., 1974); and incubated for 24 hours at 37 °C. Then returned to the incubator for at least another 24–48 hours and reexamined for the presence of slow growing bacteria. Different colonies were sub-cultured and incubated again on blood agar base and MacConkey agar until clear separated colonies were observed in a petri-plate. Then, the pure colonies were transferred to nutrient agar and allowed to grow inside incubator. The identification of bacteria were made using colony morphology,hemolytic characteristic; gram staining, catalase test, coagulase test, CAMP test and IMViC (Indole, Methyl red, Voges-Proskauer, Citrate) (Cheesbrough, 2006; Quinn et al., 2002). Additionally, the isolation of microbeswere made using selective and differential media like MacConkey agar, Bacillus cereus agar, Mannitol salt agar and purple base agar, and Eosin methylene blue agar.

Questionnaire survey

The survey was conducted using semi-structured questionnaires regarding the different potential risk factors like breed, age, housing condition, milking hygiene, general management condition and general awareness on the disease. The barns were inspected for cleanness, milking practices and for other factors associated with mastitis. Then examined clinically, using visual, the through palpation to detect possible swelling, pain, and disproportional symmetry blindness of teats and discoloration of milk for the presence of mastitis according to (Quinn *et al*., 2002).

Information on animal and farm-based risk factors was collected in two separate pre-designed questionnaires, by observation, and by interviewing of owners. A check-list was used to record such information as the cows' age, breed, parity, lactation stage, and body condition, problems of leaking milk and previous history of mastitis. Farm-based risk factors considered were teat drying, teat cleaning, floor types, teat dipping, milkers, bedding and treatment history.

Statistical analysis

The data was compiled and analyzed with STATA version 14. Prevalence estimation of commonly isolated pathogens in Sodo regional laboratory was determined using standard formulae (i.e., the number of positive animals/samples divided by the total number of animals/samples examined). Descriptive statistics such as percentages and frequency distributions was used to describe/present the nature and the characteristics of the data.

Results

Survey analysis

Based on the survey study, out of 274 respondents, 260 households were accustomed with semi-extensive and 14 households were accustomed with intensive farming system; and also out of 274respondents 154 households have no more awareness on mastitis and 120 households have awareness on mastitis how to control, way of transmission and what does mastitis mean. On another hand, 78/274 (28.5%) respondents have good farm hygiene and 196/274(71.5%) had poor farm hygiene including washing hands and udder and use of pillow to clean udder before milking.

Table 2: Farm owner's awareness about bovine mastitis in Damot gale and Damot sore districts

	Category	No. of households	Distribution
Woredas	Damot Gale	137	50.0
	Damot sore	137	50.0
awareness of mastitis	Yes	120	43.8
	No	154	56.2
Herd size	<5	198	72.3
	>5	76	27.7
management type	semi-intensive	260	94.9
	Intensive	14	5.1
bedding material	Yes	91	33.2
	No	183	66.8
teat dipping	Yes	0	0.0
	No	274	100.0
culling chronically infected cows	Yes	28	10.2
	No	246	89.8
dry cow therapy	Yes	81	29.6
	No	193	70.4
drying of udder after washing	Yes	28	10.2
	No	246	89.8
milking mastitic cow last	Yes	87	31.8
	No	187	68.2
hand prewashing	with soap	24	8.8
	without soap	250	91.2
hygiene farm	good	78	28.5
	Poor	196	71.5

Prevalence of mastitis based on different risk factors

Out of 384 milk samples collected and inspected for the presence of mastitis, 22/384 (5.7%) animals were positive for clinically mastitis and 69/384 (18%) animals were positive for sub clinically mastitis among which 48/384 (12.5%) strongly positive for CMT test and 21/384 (5.5%) sample were weakly positive for CMT test. Overall mastitis prevalence in the study area was 91/384 (23.7%). These and other factors are listed as follows in the table.

Table 3: The prevalence of mastitis in two districts

Districts	Sample no.	CMT test Positive		Prevalence		Clinically positive	Prevalence
		strong	Weak	strong	Weak		
Damot Gale	192	29	13	7.6%	3.4%	12	31%
Damot sore	192	19	8	5%	2%	10	2.6%

Table 4: Univariable logistic regression analysis of the association of cow-level mastitis with different risk factors

Variables	Examined	Mastitis +ve	Prevalence (%)	OR	95%CI	P-value
District					.3785933 - .9829643	0.042
Damot gale	192	54	28%	ref		
Damot sore	192	37	71%	0.6		
Breed					.597044 -1.690985	0.986
Local	110	26	23.6%	ref		
Cross	274	65	23.7%	1		
Parity					.6159377- 2.540474	0.536
1 to 3	54	11	20.4%	ref		
>=4	330	80	24%	1.2		
Type of management					.4074315- 1.216031	0.208
Intensive	83	24	29%	ref		
Semi-intensive	301	67	22%	0.7		
Herd size					.7603584- 1.983778	0.401
Small	238	53	22%	Ref		
Large	146	38	26%	1.2		
Manure drainage system					8.156975 - 24.95475	0.000
Good	289	31	10.7%	ref		
Poor	95	60	66.6%	14		
Use of bedding material					.3475875 .986617	0.044
No	92	29	31.5%	ref		
Yes	292	62	21.2%	0.6		

Culture growth

During CMT test strongly positive samples were culture in Wolaita sodo regional laboratory center and growth result was as follows on the table

Table 5: CMT isolated sample culture growth percentage

Test used	No. of sample	Percentage (%)
Growth on culture	35	73%
No growth on culture	13	27%

Isolated bacterial species

Bacteria species isolated from strongly CMT positive samples that growth on culture were

Table 6: The frequency of bacteria isolated from bovine mastitis study areas

Bacteria isolated	Total	Isolates frequency
S. aureus	27	44.3
Str. Agalactiae	12	19.7
Str. Dysgalactiae	8	13.1
E. coli	14	22.9

Discussion and Recommendation

This study showed the overall prevalence of mastitis in dairy cows in Damot gale and Damot sore district was 23.7%, which is comparable with 29.5% prevalence reports of Wolaitasodo (Mulugeta and Wasse, 2013) and 34.7% by Jirata and Endalem. However, it is relativelylower than the reports of 62.6% by Abebe*et al*. (Abebe et al., 2016), 52.9% by Lidet*et al*. (Lidet et al., 2013), and 73.4% by Rediet*et al*(Rediet *et al.*, 2013). On the other hand, this finding was higher than the previousfinding from5.1% by Fitsum and Mandefrot in Wolaitasodo town dairy farms (Fitsum and Mandefrot, 2017). The different reports are from different management systems, breeds of cattle and agro-climatic areas, thus these and other risk factors could contribute to the variability of mastitis prevalence among reports.

The occurrence of bovine mastitis was significantly associated with manure drainage system and type of management. Farms trends of having good manure drainage system which is keeping farm clean have odd ratio of 16 times lower risk of having mastitis than farms with poor manure drainage system. As a result revealed in this study there is statistically significant difference between intensive and semi-intensive farming system. Dairy animals in semi-intensive farming system has odd ratio of 0.5 (half) times lower risk having mastitis when compared to farms in intensive farming system. However, there is no statistically significant difference, cows with parity of more than five have two times more probability of mastitis occurrence than cows with less than five five parity. This might be due to enlargement of teat openings and size with increasing parity.

S. aureus was the predominant pathogen involved in constituting 44.3% of all bacterial isolates in the current study. *Streptococcus* species were also found prevalent with isolates of: *Streptococcus agalactiae* 19.7% and *Streptococcus dysgalactiae* 13.1% and there was also another bacteria E.coli 22.1% isolated from CMT positive sample. This finding was close relation to previous finding of

Wolaitasodo (Mulugeta and Wasse, 2013) and there is slight with previous finding from Adama town (Rediet *et al*., 2013).

There is also scarcity of knowledge and attitude on farmers in study area regarding well keeping hygiene of farm, culling chronically infected animals, awareness about mastitis disease, use of bedding material, teat dipping and hand prewashing before milking. Only 10.2% of farmers cull chronically mastitis infected animals from the farm. Only 43.8% of owners know about mastitis disease and among them only 33.2% use bedding materials, 8.8% wash hands before milking. This finding indicates there should be continuous training and/or awareness creation on mastitis disease and its economic importance and zoonotic effect in Ethiopia. This finding has close relation to previous finding ofAdama town (Rediet *et al*., 2013)

Farm owners/attendants interview showed that they had lack sufficient awareness and perception about bovine mastitis including manifestation of clinical mastitis, subclinical form and effect on quality of milk, prevention methods of the disease. Therefore, wide variety of preventive and control programs should be done through extension works by different stakeholders; also, awareness and perception of the farmers or farm attendants is pivotal for the whole process of control and prevention. Moreover, awareness creation and training for the farmers is critical to enhance production and productivity of smallholder dairy farming.

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