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Keywords: *bovine mastitis, prevalence, incidence, hawassa, wendo-genet, major pathogens.*

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Longitudinal Study of Bovine Mastitis in Hawassa and Wendo Genet Small Holder Dairy Farms

Fentaye Kassa [α], Alemu Aylate Ayano [σ], Mesele Abera [ρ] & Ashenafi Kiros [ω]

Abstract- A longitudinal study was carried out to determine the prevalence and incidence of mastitis in lactating dairy cows from November 10, 2011 to June 25, 2012 in six purposively selected smallholder dairy farms in Hawassa and Wendo-Genet districts, Ethiopia. The study was carried out through field screening surveys by California mastitis test for each quarter milk sample, followed by retesting negatives group for mastitis twice a month during the study period to estimate the incidence of mastitis. At the time bacteriological examinations to identify the causative agents from mastitis positive cows were carried out. A total of 122 milking cows were examined, out of which 77 (63.1%) and 152 (31.1%) were found positive for mastitis on the basis of California mastitis test at cow and quarter level, respectively. Out of the total quarters examined, 25 (5.12%) were recorded as a blind teats. The incidences of mastitis were seen to be 0.49 in three month study period. Slight variations in incidence rate between different farms were revealed. Higher incidence rate of 0.54 was recorded in cows kept in bad concrete in comparison to cows kept in good concrete which is 0.41. On the other hand, higher incidence rate was recorded in single and three or above parity; in early and late stage of lactation. Proportion of bacterial isolates isolated from mastitis cows showed high proportion of Staphylococcus species (52.9%) followed by Streptococcus species (23.5%), Bacillus species (9.8%), E. coli (7.8%) and Corynebacterium species (5.9%). It was concluded that there was a higher incidence and prevalence of mastitis, mainly caused by Staphylococcus species and Streptococcus species. Based on the findings, recommendations were made accordingly to concerned organs.

Keywords: bovine mastitis, prevalence, incidence, hawassa, wendo-genet, major pathogens.

I. Introduction

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to rally round the economic development of the country. It is eminent that livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter supply provide mainly the needed animal protein that contributes to the improvement of the nutritional status of the people. The total livestock population for the country is estimated to be 50.8 million cattle, 25.9 million sheep, 21.9 million goats, 1.9 million horses, 5 million donkeys, 0.3 million mules, 0.8 million camels and the total poultry population at country level is estimated to be about 42 million (CSA, 2009). In Southern Nations Nationalities and People Regional States (SNNPRS), the total cattle population is estimated at about 8.8 million. Nearly all the cattle population (98.41%) is found in rural areas while a small proportion (1.59%) is found in urban areas (CSA, 2003).

Even though Ethiopia is the most populous country in cattle than any African country; up to 1997 the per Capita milk consumption was 16 kg, which was lower than other countries in the region (Asfaw, 1997). This is partly due to the low genetic milk production potential of the indigenous zebu cattle. To increase milk production cross breeding of indigenous zebu with exotic breeds particularly with Holstein Friesian is widely practiced in the country which resulted in a larger portion of the dairy cattle population especially in urban areas to be with a high level of exotic blood. However, this market oriented dairy production, a rapidly growing system in many African countries, is subjected to diseases of intensification including mastitis and reproductive disorders (Lemma et al., 2001).

Ethiopia holds large potential for dairy development due to its large cattle population and the favorable climate for improved high yielding animal breeds (Bishi, 1998). Considering the potential if smallholder income and employment generation, development of dairy farming can make significant contribution to the poverty reduction and nutritional improvement in the country (Staal, 1996). Given the considerable potential for income and employment generation from high value dairy product (Bishi, 1998). Dairy production is a biological efficient system that converts large quantities of roughage which is the most abundant of fed to milk (Reugg, 2001). In Ethiopia where access to market dairying is preferred to meet production since it makes more efficient use of feed resource and provides a regular income to the produces. Milk is very nutritional food that is reach in carbohydrate, protein, fat, vitamin and minerals. The increase in human populations, accessibility to technology input and high demand for animal product

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purchasing power in urban center had helped the urban and per-urban dairy farm in the country to flourish (Yoseph et al., 1998).

Mastitis is one of the most important disease affecting dairy cows. It is a multi-factorial disease with worldwide distribution accounting for major economic losses in dairy cattle (DeGrave and Fetrow, 1993) which incurs serious economic losses to dairy industry. A number of previous reports from different part of the country indicated that mastitis is a serious problem in the dairy industry of Ethiopia (Bishi, 1998). Bovine mastitis can reduce milk yield, increase culling rate, incur treatment cost, occasionally result in death from sever infection (Radostitis et al., 2007).

Mastitis had been known to cause a great deal of loss or reduction of productivity, to influence the quality and quantity of milk yield, and to cause culling of animals at an unacceptable age (Singh and sigh, 1994). Most estimates have shown a 30% reduction in productivity per affected quarter and a 15% reduction in production per cow per lactation (Radostits et al., 1994). The disease generally involves interplay between management practice and infection agent. Among various infectious agents, bacterial pathogens have been known to be widely distributed in the environment of dairy cows, constituting threat to the mammary gland (Schalm et al., 1989).

Mastitis is a management related disease whose prevention and control depends among other factors on the type of management employed. If management is improved; there is a reduction in the incidence of clinical mastitis and vice versa. As with most infectious disease, mastitis risk factors depends on three components i.e. exposure to the microbes, cow defense mechanism, and environment and management factors (Suriyasathaporn et al., 2000).

Therefore, the objectives of this study were:

- To estimate the prevalence and the incidence of mastitis in the study area
- To isolate major pathogen and to identify the associated risk factors

II. Materials and Methods

a) Study Area

The study was carried out in small holder dairy farms in Hawassa and Wendo-genet. Hawassa which is the capital town of SNNPRS is located at 275 km South of Addis Ababa. It lies geographically between 4o27', and 8o 30', latitude North and 34o 21', and 39o 1', East longitude. The annual rain fall and temperature varies from 800 - 1000 mm and 20.1 - 25oC, respectively. Wondo-genet is located 264 km from Addis Ababa and 30 km from Hawassa (SZPEDD, 2001).

b) Study Population

The study population constitutes of lactating government and private owned cross breed cows found in Hawassa and Wendo-Genet small holder dairy farms. Cows are kept in exclusive stalls and provided with supplementary diets in addition to the pasture and agricultural by-products.

c) Study Design

The study was a longitudinal observational study in which all the study animals were tested at the beginning of the study period for clinical and subclinical mastitis by physical examination of the udder and by CMT (California Mastitis Test), respectively. Each farm was visited for three consequetive months at two weeks interval and screening of CMT negative cows was done throughout the study period using California Mastitis Test (CMT) to detect the presence of new cases of mastitis.

Information regarding the potential risk factors for both clinical and sub clinical mastitis such as age, parity, stage of lactation, udder injury, frequency of milking, tick infestation and hygiene of the farm were collected. To identify the major bacterial pathogens milk samples were collected from mastitic cows and subjected to bacteriological examination.

d) Sample Size and Sampling Method

From both study sites a total of 122 animals from the target population were first tested for mastitis and those negative for mastitis at the first screening was followed and checked in two weeks interval during the study period and those positive for CMT removed from the study and recorded as new case.

e) Sample Collection and Laboratory Analysis

i. *Physical Examination of the Udder and Milk*

The udder was first examined visually and then palpated to detect possible fibrosis, inflammatory swelling, and atrophy of the tissue. The size and consistency of the mammary quarter were inspected for the presence of any abnormalities such as disproportional symmetry, swelling, firmness, and blindness. In addition milk from each quarter was inspected by visual inspection for presence of any flakes, clots and color change.

f) California Mastitis Test

Every two weeks after a visit to the farm all lactating dairy cows, which are negative for subclinical mastitis at the first visit, were tested with CMT during the study period. Subclinical mastitis was diagnosed based on CMT result and the nature of coagulation and viscosity of the mixture, which show the presence, and the severity of the infection respectively (Radostits et al., 1994). CMT grades were evaluated and the results graded as 0 and 1 for negative and 2 and 3 for positive (Kerro Dego and Tareke, 2003). Then milk sample was collected from CMT positive cows for bacteriological examination.

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g) Milk Collection

The udder of the animal was thoroughly cleaned with water (Chauhan and Argawal, 2006). The teat orifice was also cleaned using cotton soaked in 70% ethyl alcohol (Quinn et al., 1999). After discarding a few streams of milk, by holding the sterile collection bottle nearly horizontal, about 3 to 4 ml milk was collected (NMC, 1990). Then the samples were labeled and transported in ice-packed cool box to microbiology laboratory of the school of Veterinary Medicine, Hawassa University.

h) Bacteriological Examination of Milk Sample

In the laboratory a loopful of the milk samples were streaked on to the blood agar base enriched with 7% sheep blood and MacConkey agar. The plates were incubated at 37oC aerobically and examined after 18 to 24 hours for the presence of fast growing organism and then returned to the incubator for at least another 24 hours and reexamined (NMC, 1990). Identification of the bacteria isolate was done according to NMC (1990) and Quinn et al (2002) Colony morphology, Gram staining, catalase test, motility test, triple sugar iron reaction, CAMP test, IMViC (Indole, Methyl red, Voges-Proskauer, Citrate) and coagulase test were conducted to identify the isolates.

III. Statistical Analysis

a) Result

From the total 122 lactating cows examined for mastitis, prevalence of 63.1% (77) and 31.1% (152) were recorded at cow level and quarter level, respectively. The prevalence at each farm levels is shown in Table 1. From 77 mastitis positive cows 33 (42.9%), 19 (24.7%), 19 (24.7%) and 6 (7.8%) cows had one, two, three and four quarters infection respectively (Table 2).

Out of the total 488 quarter examined 25 (5.1%) quarters were blind out of which 3.2% (5/156), 4.2% (3/72), 5.3% (4/76), 2.1% (1/48) and 10.7% (12/112) from HU, SOS, Biniyam, Saron and Wendo- Genet but no blind teat in Eden farm (Table 3).

Out of 45 lactating cows intially at risk for mastitis 22 (48.9%) cows had mastitis in three months period of follow up and the incidence risk of 42.9%, 33.3%, 75.0%, 50.0% and 50.0% were recorded for HU, SOS, Biniyam, Saron and Wendo-Genet farm respectively but no new infection recorded in Eden farm (Table 4).

The incidence risk (IR) and the relative risk (RR) of different risk factors is shown in Table 5.

Among 51 bacterial isolates, Staphylococcus species and Streptococcus species were the dominant isolates. The relative isolation rate of Staphlococcus species was 52.9% (27), Streptococcus species 23.5% (12), that of E. coli 7.8% (4), Bacillus species 9.8% (5) and Corynebacterium species 5.9% (3) (Table 6).

Table 1 : The Prevalence of Subclinical Mastits at Cows Level and Quarter Level

Farm Name	No. of cows examined	No. of CMT positive cows	Prevalence (%)	No. of quarters examined	No. of quarters affected	%
HU farm	39	25	64.1	156	36	23.1
SOS	18	12	66.7	72	30	41.7
Biniyam	19	11	57.9	76	30	39.5
Saron	12	6	50.0	48	15	31.3
Eden	6	5	83.3	24	11	45.8
Wendo Genet	28	18	64.3	112	30	26.8
Total	**122**	**77**	**63.1**	**488**	**152**	**31.1**

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Table 2 : The Proportion of Quarters Affected from Mastitis Positive Cows

Farm Name	No. of quarters affected & prevalence								Total	%
	1	%	2	%	3	%	4	%		
HU farm	18	54.5	3	15.8	4	21.1	0	0.00	25	32.5
SOS	2	6.1	4	21.1	4	21.1	2	33.3	12	15.6
Biniyam	1	3.0	2	10.5	7	36.8	1	16.7	11	14.3
Saron	1	3.0	2	10.5	2	10.5	1	16.7	6	7.8
Eden	1	3.0	3	15.8	0	0.00	1	16.7	5	6.5
Wendo Genet	10	30.3	5	26.3	2	10.5	1	16.7	18	23.4
Total	**33**	**42.9**	**19**	**24.7**	**19**	**24.7**	**6**	**7.8**	**77**	**100.0**

Table 3 : The Prevalence of Blind Teat in Different Farms

Farm Name	No. of quarters examined	No. of blind teat	(%)
HU farm	156	5	3.2
SOS	72	3	4.2
Biniyam	76	4	5.3
Saron	48	1	2.1
Eden	24	0	0.0
Wendo Genet	112	12	10.7
Total	**488**	**25**	**5.1**

Table 4 : Incidence Risk of Mastitis in the Study Farms from December, 2011 to April 2012

Farm Name	No. of animals initially at risk	No. of animals affected	Incidence Risk (%)
HU farm	14	6	42.9
SOS	6	2	33.3
Biniyam	8	6	75.0
Saron	6	3	50.0
Eden	1	0	0.0
Wendo Genet	10	5	50.0
Total	**45**	**22**	48.9

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Table 5 : The Incidence Risk and Relative Risk of Mastitis in Association with Selected Risk Factors during the Study Period (December, 2011 to April, 2012)

Risk factors	No. of animals initially at risk	No. of animals affected	Incidence Risk (%)	Relative Risk
Floor type				
Good concrete	17	7	41.2	
Bad concrete	28	15	53.6	1.3
Age				
≤ 5 yrs	18	10	55.6	
> 5yrs	27	12	44.4	0.8
Parity				
Single	20	12	60.0	
Two	14	3	21.4	0.4
3 and above	11	7	63.6	1.1
Stage of Lactation				
Early	14	9	64.3	
Mid	9	2	22.2	2.9
Late	22	11	50.0	1.3

Table 6 : Proportion of Bacteria Isolated from Mastitis Positive Cows

Bacterial isolates	No. of isolates	%
Staphylococcus spp.	27	52.9
Streptococcus spp.	12	23.5
Corynebacterium spp.	3	5.9
E. coli	4	7.8
Bacillus spp.	5	9.8
Total	**51**	**100.0**

IV. Discussion

The result of this study showed the prevalence of mastitis in Hawassa and Wendo-genet town to be 63.11% at cows level and 31.15% at quarter level, which is in agreement with the report of Biru (1989) in Ethiopia (63%), Workineh et al (2002) in two major Ethiopian dairies (59.7%), Tolla (1996) in South Wollo (61.11%) and Byarugaba et al (2008) in Uganda (61.3%). However, it was higher than the report of Fekadu (1995) in Caffa valley in Northern Ethiopia (39.65%), Biffa et al (2005) in Southern Ethiopia (34.9%), Kerro Dego and Tareke (2003) in Southern Ethiopia (40.40%), but lower than the report of Mekibib et al (2010) in Holeta town in Centeral Ethiopia (71.05%). The overall quarter prevalence of mastitis was 31.15%. This finding is comparable with the report of Sori et al (2005) which was (30.95%). However, this finding was higher than that of Vecht et al (1989) in the Netherland and Hoyer et al (1991) in Zimbabwe, who reported 10%, 14%, respectively. This variation in the prevalence of bovine mastitis between different reports could suggest the complexity of the disease which involve interaction of several factors, mainly management practice, husbandry system, environment and factors related to causative agent and variation in veterinary service coverage.

Out of 488 quarter examined, 5.12% were blind, which may be an indication of serious mastitis problem on the herd and lack of screening tests and treatment of subclinical mastitis , and inadequate follow up chronic mastitis were considered to be the major reason for the development of quarter blindness (Biffa, 2005).

In this study there was one cow with clinical mastitis but the rest of the cases were subclinical. This may be due to ease of detection of clinical mastitis and treatment of only clinical cases. Moreover absence of dry cow therapy in all farms might be the cause of higher prevalence of subclinical mastitis in the study area.

The overall incidence risk mastits in the three months period of follow up is 0.49, this finding is in agreement with the report of Roy et al (1999) in India which had the incidence of 0.47. This result was higher

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than the report of Bartlett et al (1991), Rajala et al (1999), Barkema et al (1998), Lucey et al (1996) which were 0.097, 0.18, 0.24 and 0. 26 respectively.

In this study relatively higher incidence risk (0.75) was recorded in Binyam farm when compared with other farms. This might be due to improper washing of hands and teats before milking and use of one towel for each cow. According to Rice and Bodman (2004) sanitary milking habits are important to avoid the spreading of bacteria or their proliferation.

The result of the present study also revealed higher incidence of mastitis (0.54) in cow kept under bad concrete as compared to cows kept in good concrete (0.41). The relative risk of mastitis in cows kept under bad concrete is 1.3 times higher than that cows kept in good concrete. This might be due to the favorable environment created for survival and multiplication of bacterial pathogens.

In this study there were higher risk of mastitis in cows with single parity (0.60) and cows with more than two parity (0.64) when compared with cows with two parity (0.21). However studies conducted by Houben et al (1993), Lucey et al (1994) and Rajala et al (1999) showed incidence risk of 6.6%, 17%, 12.1% for parity one, 9.0%, 22%, 14.3% for parity two and 14.2%, 24%, 14.9% for parity three respectively.

The finding of this study also showed higher incidence risk of mastitis in early (0.64) and late (0.50) stages of lactation as compared to mid (0.22) stage of lactation. The finding of higher incidence risk in early stage of lactation is in agreement with Barkema et al (1998). This increase in incidence risk of mastitis at early stage might be due to high milk yield at this stage of lactation (Bartlett et al., 1991; Grohn et al., 1995; Smith et al., 2000).

The finding of higher proportion of Staphylococcus species (52.9%) from total isolates is in consistent with that of Wokineh et al (2002) who reported 57% of the total bacterial isolates. The finding of higher proportion of Staphylococcus species might be due to lack of effective udder washing and drying, post milking teat dip and drying and hand washing (Radostits et al.,1994). It is also attributed to the wide distribution of the bacteria on the skin of teats and udder (Mac Donald, 1997).

The staphylococci have adapted to survive in the udder, they usually establish chronic, subclinical, infection and are shed in the milk which serves as a source of infection for other health cows during the milking process (Radostits et al., 1994).

In this study Streptococcus species accounted for 23.5% of the total isolates next to Staphylococcus species. This finding was in agreement with Zerihun (1996) which was (27%), but lower than the report of Kingwill et al (1991) which was (80.95%), Tolosa (1987) (53.55%). The lower isolation rate of Streptococcus species might be due to wide spread use of penicillin in the area for the treatment of mastitis. The finding of E. coli in this study may be associated with poor hygienic condition in the farm, unimproved management, wet and muddy stall were cows are kept.

V. Conclusion and Recommendation

Generally, mastitis is one of the complex diseases of dairy cows which involve an interaction between management practice and infectious agent occurring throughout the world. The disease has also been reported in different parts of Ethiopia with varying prevalence. But incidence of mastitis in the country has not been studied like that of prevalence and the spreading of mastitis among cows was not determined. Inadequate hygienic condition of dairy cow's stalls, poor milking procedure, poor animal health service and lack of proper attention to the heath of the mammary gland were important for the high prevalence and incidence of mastitis in the study area. The present study showed that the occurrence of mastitis at cow and quarter level is high and the incidence of mastitis also high, which can interfere with efficiency of milk production and has high economic importance. From this study the most important pathogens causing mastitis in the study area was Staphylococcus species and Streptococcus species, which were isolated from the milk sample in higher proportion, which is associated with unhygienic milking practice and poor herd management, whereas the finding of other bacteria such as E. coli is associated with poor hygiene of the stall and bedding.

Based on this study, to reduce the incidence risk of mastitis the following recommendations are forwarded:

- ✓ Standard milking procedure, such as pre and post milking udder washing should be applied to reduce the risk of transmission.
- ✓ Regular screening of subclinical mastitis for early detection and treatment, and culling of chronically infected cows should be practiced.
- ✓ Dry cow therapy should be applied to reduce the occurrence of new infection after parturition.
- ✓ Adequate housing with proper sanitation of the cow's barn and bedding to reduce environmental mastitis.
- ✓ To reduce the prevalence of the disease, different epidemiological factors that interplay in mastitis occurrence should be studied routinely.

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