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Full Length Research Paper

Major causes of mastitis and associated risk factors in smallholder dairy cows in Shashemene, southern Ethiopia

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A cross-sectional study was conducted from October 2010 to May 2011 to estimate the prevalence of mastitis, isolate and characterize major bacterial pathogens and to assess the association of some putative risk factors with occurrence of mastitis in cows in smallholder dairy farms in Shashemene, southern Ethiopia. A total of 245 lactating cows (111 Holstein, 98 Holstein-local Zebu crosses and 36 indigenous Zebus) were examined clinically and California Mastitis Test (CMT) was used to detect clinical and subclinical mastitis. The overall prevalence of mastitis at cow and quarter level was 37.1% (91/245) and 30.0% (288/960), respectively. Seventy (28.6%) cows were with subclinical mastitis while only 20 (8.6%) had clinical mastitis. The prevalence of mastitis significantly (P<0.05) differed with breed, parity, stage of lactation and previous record of mastitis. A total of 217 bacterial isolates were recovered from 288 mastitic milk samples and Gram-positive cocci were the most common pathogens. The pathogens isolated were *Staphylococcus aureus* (28.1%), other *Staphylococcus* species (22.1%), *Streptococcus agalactiae* (10.1%) other *Streptococcus* species (14.3%), coliforms (22.1% that is *Escherichia coli*, 10.6%; *Klebsiella* species 7.8% and *Enterobacter* species 3.7%), *Micrococcus* species (1.4%), *Pseudomonas* species (1.4%) and *Bacillus* species (0.5%). Therefore, culling of older cows with repeated mastitis records and dry cow therapy will be practiced to reduce the risk of mastitis. Culling of old and chronically affected cows, screening for mastitis, awareness creation among smallholder farmers about the importance of sub-clinical mastitis and milking and barn hygiene should be considered in reducing the effect of mastitis.

Key words: Bacterial isolates, California Mastitis Test, mastitis prevalence, risk factors, Shashemene.

INTRODUCTION

In Ethiopia, livestock represents a major national resource and form an integral part of agricultural production system. Cows represent the largest population of cattle production of the country; 42% of the total cattle are milking cows. Per capita consumption of milk in Ethiopia is as low as 17 kg per head while the average figure for Africa is 26 kg per head (Gebrewold et al., 2000). The quality and quantity of milk production deteriorate due to biological causes including the low genetic potential of the animals, poor nutrition and prevalence of diseases (Atyabi et al., 2006). Mastitis is among the factors contributing to reduced milk production (Biffa et al., 2005), and it is among the most important diseases in dairy animals with worldwide distribution (Zhao and Lacasse, 2007). Mastitis is characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue (Radostits et al., 2007). Mastitis has been known to cause a great deal of loss or reduction of productivity. It influences the

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quality and quantity of milk, and causes culling of animals at an unacceptable age (Mungube et al., 2005).

Several bacterial pathogens are implicated in bovine mastitis. From epidemiological and pathophysiological standpoint the pathogens are regarded as contagious, teat skin opportunistic or environmental (Radostits et al., 2007). Although there are studies carried out so far by different researchers on the prevalence, risk factors and the major bacterial causes of bovine mastitis in Ethiopia including the present study area, it is necessary to update the information as the epidemiology of diseases including mastitis is dynamic. Therefore, this study was conducted to estimate the prevalence, to identify the associated risk factors and to isolate and characterize major bacterial causes of mastitis in dairy cows in Shashemene town, southern Ethiopia.

MATERIALS AND METHODS

Study area and animals

The study was carried out in Shashemene town, west Arsi zone of Oromia Regional State, Ethiopia. Shashemene is located 250 km south of Addis Ababa, at an altitude of 1920 to 1947 m a.s.l. The study involved a total of 245 randomly selected lactating cows (111 Holstein, 98 Holstein-Local Zebu crosses, and 36 indigenous Zebus) and 960 quarters from smallholder farms. All the study cows were hand milked, and milked twice a day. There was no tradition of record keeping in the study farms.

Study design and sampling

The study employed was a cross-sectional study type. The sample size was determined based on the formula given by Thrusfield (2007) considering 5% absolute precision, 95% level of significance and expected prevalence of 20%. Risk factors considered were age, parity, stage of lactation, breed of cow, record of mastitis, floor type and bedding. Questionnaires and direct observations of the farms were used to collect information regarding the risk factors used in the analyses.

Physical examination of udder and milk

The udders of the study cows were examined visually and by palpation for presence of clinical mastitis. During examination attention was paid to cardinal signs of inflammation, symmetry, size and consistency of udder quarters. In addition the milk was examined for any abnormalities using strip cup (Radostits et al., 2007).

California mastitis test

The California mastitis test (CMT) was conducted to diagnose the presence of subclinical mastitis and it was carried out according to procedures given by Quinn et al. (1999). Based on the thickness of the gel formed by CMT reagent and milk mixture, test results were scored as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). Milk samples with test result of CMT 1 to 3, were classified as evidence of subclinical mastitis (Radostits et al., 2007; Quinn et al., 1999).

Sample collection and bacteriological examination

Ninety one (91) quarter milk samples, one from each mastitic cow (clinical and subclinical), were collected for bacteriology. Milk samples were collected after the skin of the udder was washed with tap water and dried with clean towel and the teat ends were swabbed with cotton soaked in 70% ethyl alcohol. The samples were collected in sterile universal bottles held nearly horizontal, after discarding the first few squirts of milk (Quinn et al., 1999). Milk samples were immediately transported to the microbiology laboratory of the School of Veterinary Medicine of Hawassa University in ice packed cool box for bacteriological analysis.

All milk samples were subjected to bacteriological examination according to Quinn et al. (1999) commencing on the date of collection. A loop full of milk sample was streaked on blood agar base enriched with defibrinated sheep blood and MacConkey agar using the quadrant streaking method. Then the plates were incubated aerobically at 37°C for 24 to 48 h and examined for growth, morphology, pigmentation and haemolytic characteristics of the colonies. Subcultures were done to obtain pure isolates for further identification. Identification was done according to the standard methods described by Quinn et al. (1999).

Data analysis

The response variable considered in the analysis of our data was mastitis status of a cow and the potential risk factors considered were parity of the cow, stage of lactation, breed, age, previous mastitis history and floor type. Prevalence was calculated as a percentage value and univariable logistic regression analyses was used to assess the association between mastitis and individual risk factors considered in the study. STATA version 11.0 (Stata Corp College Station, TX) was used for all types of analyses.

RESULTS

Prevalence

The prevalence of mastitis at cow and quarter level was 37.1% (91/245) and 30.0% (288/960), respectively. From the total of 245 lactating cows examined 21 (8.6%) had clinical and 70 (28.6%) had subclinical mastitis, while the prevalence at quarter level for clinical and subclinical mastitis was 6.7 and 23.3%, respectively (Table 1). Out of the total 245 cows and 980 quarters examined 17 (6.9%) cows and 20 (2.0%) quarters had blind teats. Ten of the blind teats were of hind quarter and the remaining 10 of front quarters. The detail of the number of cows with number of affected quarters is shown in Table 2. Most cows with mastitis had all their 4 quarters affected.

Risk factors

Risk factors logistic regression analyses showed that

Table 1. Prevalence of clinical and subclinical mastitis at cow and quarter level.

Type of mastitis	Cow level (n=245)		Quarter level (n=960)	
	Number positive	Prevalence (%)	Number positive	Prevalence (%)
Clinical	21	8.6	64	6.7
Subclinical	70	28.6	224	23.3
Total	91	37.1	288	30.0

Table 2. Frequency and percentage of cows by the number of mastitis affected quarters (n=245).

Number of quarter affected	Clinical		Subclinical		Total	
	Number of cow	%	Number of cow	%	Number of cow	%
One	4	1.6	8	3.3	12	4.9
Two	3	1.2	12	4.9	15	6.1
Three	2	0.8	8	3.3	10	4.1
Four	12	4.9	42	17.1	54	22.0
Total	21	8.6	70	28.6	91	37.1

Table 3. Regression analysis of various risk factors association with occurrence of mastitis.

Factor	Group	Number examined	Card positive	Prevalence (%)	Odds ratio (95% CI)	P- Value
Parity	1 – 2	36	8	22.2	1	0.0026
	3 – 6	87	25	28.7	1.41 (0.57 – 3.52)	
	≥ 7	122	58	47.5	3.17 (1.34 – 7.51)	
Stage of lactation	Early (1–3m)	83	41	49.4	2.30 (1.32-4.00)	0.0107
	Mid (4–7m)	131	35	26.7	1	
	Late (> 7m)	31	15	48.4	2.0 (0.57 – 6.76)	
Breed	Local zebu	36	6	16.7	1	0.0023
	Cross	98	33	33.7	2.54 (0.96 – 6.71)	
	Holstein	111	52	46.8	4.41 (1.70 – 11.42)	
Age	3 – 5 years	50	14	28.0	1	0.0010
	6 – 8 years	105	30	28.6	1.03 (0.49 – 2.17)	
	> 8 years	90	47	52.2	2.81 (1.34 – 5.91)	
Mastitis record	Absent	216	68	31.5	1	0.0000
	Present	29	23	79.3	8.34 (3.25 – 21.43)	
Floor	Concrete	150	46	30.6	1	0.0086
	Soil	95	45	47.4	2.03 (1.20-3.46)	
Overall		245	91	37.1		

parity, stage of lactation, breed, age, previous record (exposure) of mastitis and floor type had significant effect (P<0.05) on the prevalence of mastitis. The logistic regression analyses of risk factors outcome is shown in Table 3. As expected, old cows more than 8 years old had the highest prevalence of mastitis (P<0.01); likewise prevalence of mastitis was significantly high in cows with parity 7 and above (P<0.01). Cows in their early and late stage of lactation suffered most from mastitis compared to cows in their mid stage of lactation (P<0.05). Holstein

Table 4. Frequency and proportion of bacterial species isolated from clinical and subclinical mastitis (number of isolates=217).

Bacterial isolate	Clinical		Subclinical		Total	
	Number	%	Number	%	Number	%
Staphylococcus aureus	10	4.6	51	23.5	61	28.1
Other *Staphylococcus* species	8	3.7	40	18.4	48	22.1
Streptococcus agalactiae	5	2.3	17	7.8	22	10.1
Other *Streptococcus* species	5	2.3	26	12.0	31	14.3
Escherichia coli	12	5.5	11	5.1	23	10.6
Klebsiella species	8	3.7	9	4.2	17	7.8
Enterobacter species	3	1.4	5	2.3	8	3.7
Micrococcus species	0	0.0	3	1.4	3	1.4
Pseudomonas species	0	0.0	3	1.4	3	1.4
Bacillus species	0	0.0	1	0.5	1	0.5
Total	51	23.5	166	76.5	217	100.0

cows had significantly higher (P<0.01, OR=4.4) prevalence of mastitis than local zebu cows. Cows with previous history of mastitis had higher mastitis prevalence (P<0.001) compared to cows with no previous history of mastitis. Floor type had significant effect (P <0.01) on prevalence of mastitis, too. Cows kept in houses with soil floor had higher prevalence than cows managed on concrete floor.

Bacterial isolates

Table 4 shows the bacterial isolates from 91 mastitic cows. Milk samples from all 91 mastitic cows (both clinical and subclinical) were cultured and a total of 217 bacteria were isolated. The most frequent isolates were *Staphylococcus aureus* (28.1%), other *Staphylococcus* species (22.1%), *Streptococcus agalactiae* (10.1%), other *Streptococcus* species (14.3%), coliforms (*Escherichia coli, Klebseilla* species and *Enterobacter* species) (22.1%), *Micrococcus* species (1.4%); *Pseudomonas* species (1.4%) and *Bacillus* species (0.5%). It appears that gram positive cocci such as *S. aureus*, *S. agalactiae* and other *Staphylococcus* and *Streptococcus* species were more involved in subclinical mastitis, while agents of environmental mastitis (*E. coli, Klebsiella, Enterobacter*) were more or less equally involved in both subclinical and clinical mastitis.

DISCUSSION

Prevalence

The overall prevalence of 37.1% at cow level and 30% at quarter level is comparable to the findings of Biffa et al. (2005), Kerro and Tareke (2003), Workineh et al. (2002), Bishi (1998) and Fekadu (1995) who reported 34.9% in Southern Ethiopia, 40.4% in southern Ethiopia, 38.2% in Adami-Tulu central Ethiopia, 39.8% in and around Addis Ababa and 39.7% in Chaffa valley in north eastern Ethiopia, respectively. However, it is relatively lower than the report of Mekibib et al. (2010), Lakew et al. (2009), Sori et al. (2005) and Mungube et al. (2004) who recorded 71.1% from Holeta, 64.6% from Assela, 52.8% from Sebeta and 46.6% from central highlands of Ethiopia, respectively. Prevalence of subclinical mastitis (28.6%) was higher than that of clinical mastitis (8.6%) in the present study, which is in general agreement with several earlier reports from different parts of Ethiopia (Abera et al., 2010; Mekibib et al., 2010; Lakew et al., 2009; Almaw et al., 2008; Getahun et al., 2008; Biffa et al., 2005; Mungube et al., 2004; Kerro and Tareke, 2003; Workineh et al., 2002) and elsewhere in Africa (Kivaria et al., 2004).

Risk factors

Risk of mastitis increased with age and parity. This observation is in agreement with the reports of Abera et al. (2010), Biffa et al. (2005), Mungube et al. (2004) and Kerro and Tareke (2003) from the country. Mastitis prevalence was high in early and late stage of lactation. This result is consistent with observations of Biffa et al. (2005) and Kerro and Tareke (2003) who reported high prevalence of mastitis in the early and late stage of lactation. Breed showed significant influence on the prevalence of mastitis. The observed high prevalence of mastitis in Holstein Friesian compared to local cows is in agreement with the findings of Biffa et al. (2005), Girma (2002) and Biru (1989). As stated in Radostits et al. (2007) this may be associated with differences in anatomical and physiological characteristics of the mammary gland, as well as high milk yielding of the cows.

The association between soil floor and high prevalence of mastitis recorded in our study is consistent with the findings of Abera et al. (2010). Earlier works implicated poor barn hygiene to high prevalence of mastitis (Sori et al., 2005). Cows with previous history of mastitis were found more likely to be mastitic. This observation is supported by the findings of Biffa et al. (2005). This finding suggests that treatment of cows for mastitis may not be effective in eliminating the pathogens and the disease may be carried over from previous lactations to next lactation. Also, there are reports of antimicrobial resistance among pathogens which cause mastitis in Ethiopia (Abera et al., 2010).

Bacterial isolates

Staphylococci and *Streptococci* species together accounted for 50.2% of the total isolates, while *Staphylococci* alone were 24.4% of the isolates. These bacteria were implicated as the most frequently isolated from mastitic milk in Ethiopia: *Staphylococci* and *Streptococci* species accounted for 73.5% (Workineh et al., 2002), 63.0% (Kerro and Tareke, 2003), 73.2% (Sori et al., 2005), 89.0% (Almaw et al., 2008) and 57.2% (Mekonnen and Tesfaye, 2010) of the total isolates of bacteria from mastitic milk. The high prevalence of *Staphylococci* and *Streptococci* may be partly explained by presence of these agents on the skin and mucus membranes of various parts of the animal body (Carter and Wise, 2004; Quinn et al., 1999) and their contagious nature, especially *S. aureus* and *S. agalactiae* (Radostits et al., 2007).

Moreover, *Staphylococci* survive in the environment for a prolonged period (Quinn et al., 1999). Coliforms (*E. coli*, *Klebsiella* and *Enterobacter*) were the third most commonly isolated bacteria (22.1%) to *Staphylococci* and *Streptococci* which is consistent with Kerro and Tareke (2003) and Mekonnen and Tesfaye (2010). Because these bacteria are environmental pathogens their occurrence may be associated with poor quality management of housing and bedding (Radostits et al., 2007).

Conclusion

The present study revealed that mastitis is still prevalent in smallholder dairy farms in the study area, and further confirms that the subclinical form is the most prevalent. Mastitis prevalence was associated with several risk factors. The study also concludes *Staphylococci* and *Streptococci* are the most important causes of bovine mastitis, especially subclinical mastitis, in smallholder dairy farms.

Culling of old and chronically affected cows, screening of cows and milk for clinical and subclinical mastitis, dry cow therapy, hygiene at milking and cow house hygiene should be considered in attempts to reduce prevalence of mastitis. Moreover, extension services and training programs aiming at creation of awareness about the importance and prevention of subclinical mastitis among smallholder dairy farmers is recommended.

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