Prevalence and Etiology of Subclinical Mastitis in Small Ruminants of Tigray Regional State, North Ethiopia

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Abstract

A study was conducted at Kafta Humera and Tanqua Abergelle *Districts* from April to June, 2011 to assess the prevalence of subclinical mastitis in lactating small ruminants and identify bacterial causative agents. A total of 390 lactating animals comprising 255 goats and 135 sheep were randomly selected from population and screened for evidence of subclinical mastitis. The overall prevalence of subclinical mastitis was found to be 18.03% (46/255) and 28.14% (38/135) in goats and sheep, respectively. California mastitis test (CMT) positive milk samples were subjected to bacteriological examination and the following bacteria were isolated; coagulase negative Staphylococcus (44.7%), *Staphylococcus aureus* (27.7%), *Escherchia coli* (17.0%) and *streptococc*i (10.63%). Risk factors; species ($p = 0.021$), study site ($p < 0.001$), sheep breed ($p = 0.018$) and goat breed ($p = 0.009$) showed statistically significant association with the occurrence of subclinical mastitis in small dairy ruminants. However, there was no statistically significant association between risk factors such as Age ($p = 0.779$), parity ($p = 0.201$) and stage of lactation ($p = 0.952$). Therefore, it can be concluded that mastitis is a significant disease in small ruminants that affects their productivity and measures need to be taken to control the disease.

Key words: Bacteria; CMT test; Prevalence; Small ruminants; Subclinical mastitis

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Introduction

Small ruminant (sheep and goats) rearing provide vast range of products and services such as immediate cash income, meat, milk, skin, manure, risk spreading/ management and social functions (Adane and Girma, 2008) for poor farmers in many parts of Ethiopia. Small ruminants with their higher reproductive capacity and growth rates are ideally suited to production by resource-poor smallholders (Tibbo, 2006). According to a recent report by Central Statistics Authority of Ethiopia, there are about 26.1 million and 21.7 million sheep and goats' population heads in Ethiopia, respectively (CSA, 2008). The small ruminant population of Tigray Region of north Ethiopia is estimated to be 1,022,779 sheep and 1,588,779 goats (TBoARD, 2006).

Though small ruminant production is playing an important role in the improvement of the incomes for poor farmers, poverty and hunger alleviation and can contribute a major role in the country developmental plan, small ruminant production and productivity and producers' benefits are far below expectations due diseases, malnutrition, low genetic potential and poor management practices. Among others infectious diseases are the major constraints to small ruminant production in Ethiopia (Getahun, 2008; Gizaw *et al.,* 2010). Among these diseases mastitis is one of the important diseases affecting the productivity of small ruminants.

The nutritional components that make milk an important part of the human diet also support the growth of pathogenic microorganisms coming from milk contamination or from animal

infections (Santos *et al.,* 2009). The major income of dairy sheep and goats comes from milk production, which is mainly processed into fermented products and cheese. Thus, factors influencing milk quantity and quality, such as subclinical mastitis, have an overwhelming effect on economic losses to the farmer. In recent studies it was demonstrated that curd yield and its properties were damaged by subclinical mastitis as well, thus posing a secondary loss to the dairy industry (Leitner *et al.,* 2004a, b). Compared to clinical mastitis, the subclinical form has major economic significance as this form of the disease doesn't manifest clinical symptoms (Quinn *et al.,* 2002).

In Ethiopia, there is paucity of literature dealing with the prevalence of small ruminant mastitis. Only few studies reported the prevalence of the disease in the country (Assefa *et al.,* 2006; Megersa *et al.,* 2009); making information available on the prevalence of the disease and associated economic loss inadequate. However, sheep and goat milk is routinely consumed by the rural and semi-urban societies of Ethiopia next to the cow's milk. This gap of information is higher in Tigray region in spite of the existence of huge number of sheep and goats of different breeds; and this limitation can pave a good opportunity for the transmission of different zoonotic diseases from animals to humans. Thus, the present study is designed to assess the prevalence of subclinical mastitis in small ruminants (lactating goats and sheep), to determine the association of risk factors with the occurrence of subclinical mastitis and to isolate and identify the major bacterial causative agents of subclinical mastitis.

Materials and Methods

Study Area: This study was conducted on lactating Begayt and Abergelle sheep and goats breeds of Kafta Humera and Tanqua Abergelle *districts,* respectively. Tanqua Abergelle *District* is located in the central zone of Tigray regional state while Kafta Humera is located in Western zone of Tigray Region of Northern Ethiopia. The total sheep and goats population in Tanqua Abergelle district is estimated about 264,596 and 78,245, respectively while in Kafta Humera district the sheep and goat population is about 65,108 and 85,073, respectively (TBoARD, 2006).

Study Design and sampling strategy: A cross sectional study was conducted to assess the prevalence of subclinical mastitis in small ruminants (sheep and goat). A total of 390 lactating, Begait and Abergelle breeds of goats and sheep were included in the study. The sample size for the current study was determined according to the formula given by Thrustfield, (2005) for cross-sectional, random sampling method. A 5% absolute precision and 95% level of confidence was used for determining the sample size. An expected prevalence of 20% was used to determine the maximum sample size. Accordingly, the sample size was calculated to be 245 for each species however, a total of 255 lactating sheep and 135 lactating goats were included in this investigation.

California Mastitis Test (CMT): The California mastitis test was conducted to diagnose the presence of subclinical mastitis. This screening test was performed according to the procedure given for mastitis by Quinn et al. (2002). The result was scored as 0, +1, +2 or +3 depending on the intensity of reaction. Samples with CMT result score of 0 and +1 were considered as negative, while those with score of +2 or +3 were taken as positive. If at least one quarter was positive by the CMT the lactating goat or sheep was considered positive.

Milk sample collection: Milk samples were collected aseptically from quarter's diagnosed with CMT > +2 and were submitted for bacteriological examination. Briefly, the udder of the goat/sheep was thoroughly washed with water and dried with clean towel. After disinfecting the teats with 70% ethyl alcohol swabs, milk was collected. The first 3-4 streams of milk were discarded and then 5-10 ml of milk was collected from each teat aseptically in separate universal bottles held a slightly horizontal position in order to avoid contamination from the udder (Singh et al., 2007). Tubes were sealed properly and transported on ice to Veterinary

Table-1. CMT results of milk samples of sheep and goats collected from Kafta Humera and Tanqua-Abergelle

Species	Sampled animals (N)	CMT positivity		
		Single halve	Both halves	Total
Study site - Kafta Humera				
Caprine	76	12 (16.2%)	9 (11.8%)	21 (27.6%)
Ovine	74	20 (27.0%)	7 (9.4%)	27 (36.5%)
Study site - Tanqua-Abergelle				
Caprine	180	23(12.8%)	2(1.1%)	25 (13.9%)
Ovine	60	10(16.7%)	1(1.7%)	11 (18.3%)

Microbiology laboratory in Mekelle University, where samples were immediately cultured or kept in refrigerator at 4°C for a maximum of 24 h until cultured on standard bacteriological media.

Bacteriological examination of milk samples: Bacteriological examination was done on a total of 23 goat and 24 sheep CMT positive milk samples collected from the study sites according to Quinn *et al.* (2002). A loopful of milk sample was streaked on tryptose blood agar base enriched with 7% defibrinated sheep blood (Oxoid, UK) and MacConkey agar (Oxoid, UK) plates using the quadrant streaking method. Both agar plates were incubated aerobically at 37 °C for 24 - 48 h and examined for characteristic bacterial colonies. Pure culture colonies were selected and sub cultured on general purpose medium, nutrient agar (Oxoid, UK), and incubated aerobically at 37 °C for 24 - 48 h for further biochemical identification. After this incubation on nutrient agar (Oxoid, UK), bacteria were identified according to their Gram reaction and morphology. Further identification of the organisms was done by implementing biochemical tests, Catalase, Oxidase, CAMP test, IMViC tests, Triple sugar iron agar test, Nitrate reduction and Urease test. In addition mannitol salt agar was used to differentiate *S. aureus* from coagulase negative *Staphylococcus spp.*

Data collection and statistical analysis: Structured data handling format was prepared and every important information (variable) associated with the overall objective of the investigation was properly gathered and recorded. Age, breed, parity, site and length of lactation were recorded. Age of the study animals was recorded from the owners' information and dentition characteristics (Oltenacu *et al.*, 1999).

Data obtained both from CMT test and data handling format were stored in Microsoft Excel spread sheet (Microsoft Corp.). Prevalence of mastitis related to specific risk factors was determined as the proportion of affected sheep/goat out of the total examined. These data were analyzed using Statistical analysis system (SAS) version 5 statistical software. To answer the question of whether or not there is a significant association between all the risk factors, we conducted chi-square (χ^2) test using the cross tabulation feature of the software. Logistic regression analysis and odds ratio (OR) was done to see the strength of association. $P < 0.05$ was taken as statistically significant.

Results

Prevalence of Subclinical Mastitis: The 390 sampled animals from the two study sites for the current study comprise 255 lactating goats and 135 sheep. Out of this population, 46/255 (18.0%) goats and 38/135 (28.1%) sheep showed CMT test positivity. Detail information of the CMT result of the overall sampled animals from the two study sites is indicated in Table 1.

Association of Risk Factors with the Occurrence of Subclinical Mastitis: During the study period, certain potential risk factors such as study site, age, species, breed, parity and length of lactation were taken in to consideration to see the integration with the occurrence of subclinical mastitis in the different species and breeds of the lactating small ruminants of the two study sites. Among the risk factors; species ($p = 0.021$), study site ($p < 0.001$), sheep breed ($p = 0.018$), goat breed ($p = 0.009$) showed statistically significant association with the occurrence of subclinical

Table-2. Univariable logistic-regression analyses (LR) of risk factors for the occurrence of subclinical mastitis in small dairy ruminants of Kafta Humera and Tanqua-Abergelle, Northern Ethiopia, OR-Odds Ratio, CI-Confidence Interval

Risk factors	Category level	N	Prevalence N (%)	Univariable LR analyses results			
				P-value	OR	95% CI of OR	
						Lower	Upper
Species	Ovine	135	38 (28.1)	0.021	1.780	1.088	2.913
	Caprine	255	46 (18.0)				
Study site	Kafta Humera	150	48 (32.0)	<0.001	2.667	1.628	4.367
	Tanqua-Abergelle	240	36 (15.0)				
Sheep breed	Begait	73	27 (37.0)	0.018	2.415	1.073	5.436
	Abergelle	62	11 (17.7)				
Goat breed	Begait	76	21 (27.6)	0.009	2.352	1.220	4.536
	Abergelle	179	25 (14.0)				
Age	< 4 years	191	40 (20.9)	0.779	0.933	0.576	1.513
	> 4 years	199	44 (22.1)				
Stage of Lactation	Early	223	48 (21.5)	0.952	0.973	0.567	1.675
	Mid	141	31 (22.0)				
	Late	26	5 (19.2)				
Parity	1-3	228	56 (24.6)	0.201	1.503	0.867	2.615
	4-6	146	26 (17.8)				
	> 6	16	2 (12.5)				

Table-3.The relative prevalence rates of various bacteria isolated from subclinical mastitis of goats and sheep.

Groups of bacteria	Frequency and percentage				
	Begayt goats	Abergele goats	Begayt sheep	Abergele sheep	Total
Streptococci	2 (40.0)	2 (40.0)	0 (0.0)	1 (20.0)	5 (10.6)
Staphylococcus aureus	2 (15.4)	1 (7.7)	8(61.5)	2(15.4)	13 (27.7)
CNS	4 (19.0)	6 (28.6)	8 (38.1)	3 (14.3)	21 (44.7)
E. coli	3 (37.5)	0 (0.0)	2 (25.0)	3 (37.5)	8 (17.0)
Total					47

mastitis in small dairy ruminants. Accordingly, higher rate of prevalence was recorded in ovines than caprines, Kafta Humera than Tanqua-Abergelle, Begait breeds of sheep and goats than Abergelle breeds. However, there was no statistically significant association between risk factors such as age ($p = 0.779$), parity ($p = 0.201$) and stage of lactation ($p = 0.952$). Table 2 summarizes the association of the different risk factors with the occurrence of subclinical mastitis in small dairy ruminants.

Relative occurrence of bacterial isolates: The standard primary and secondary bacterial identification procedures conducted on the CMT positive milk samples of dairy goats and sheep has revealed the isolation of four common bacterial organisms. Coagulase negative staphylococcus (CNS) were the most prevalent pathogens with prevalence rates of 44.7% followed by *Staphylococcus aureus* (27.7%), *E. coli* (17.0%), and the least isolated bacteria were *streptococci* with prevalence of 10.6% (Table 3).

Discussion

Small ruminant mastitis is an important disease that affects the productivity of the sector. Inflammation of the mammary gland (mastitis) in sheep is predominantly subclinical (Contreras *et al.*, 1997). The occurrence of clinical and subclinical mastitis in the different breeds of sheep has been investigated in various parts of the world (Al-Majali *et al.*, 2003).

The prevalence of subclinical mastitis in goats (18.0%) reported in this study is in close agreement with previous findings of Megersa *et al.* (2009) who reported 15.5% prevalence in Borana, South Ethiopia. However, the present finding in goats is lower than the prevalence reported by other workers in Ethiopia (40.9% by

Assefa *et al.* (2006) in Oromia Region) and Tanzania (e.g. 51.5% by Swai *et al.* (2008), 76.7% by Mibilu *et al*. (2007)). According to the current investigation, the a total prevalence of subclinical mastitis in lactating sheep was found to be 28.1% and this is in close agreement with the results reported in other countries of the world like 23.1% in Scotland by Berriatua *et al*. (2001) and it is significantly higher than 9.23% prevalence reported by Beheshti *et al.* (2010) in Iran. These variations could be as a result of the variations in the breed, husbandry of the animals and agroclimatical conditions.

Coagulase negative staphylococcus was the most predominant isolate in our study which accounted for about 47.7% of the bacterial isolates. This finding is in accordance with the literature and previous reports (Adwan *et al.,* 2005; Bergonier *et al.,* 2003; Leitner *et al.,* 2004b) . *Staphylococcus aureus* is the second predominant isolate in our study and Same is recorded by Beheshti *et al.* (2010) in Iran. The presence of coliform mastitis (*E. coli*) could probably be due to poor hygienic conditions, as these organisms originate from the animals environment and infect the udder via the teat canal (Quinn *et al.,* 2002).

In this study, ewes were more affected with subclinical mastitis than caprines. This great difference in the prevalence of the disease could be due to species variation which can lead to difference in their resistance and susceptibility to mastitis. In goats, there are shorter (or even no) dry period (Bergonier *et al.,* 2003); the teat of sheep is contaminated with waste product like faces and these could be the probable factors which contribute for the higher incidence of the disease in sheep. There was also variation in the incidence of the disease between the two breeds of sheep and goat, and this difference could be associated with genetic resistance of the breeds and the nature of the udder and teats. Teats of Begait breeds are pendulous and highly close to the ground. This type of teats and udder can easily be exposed to mechanical injuries; as a result variety of pathogenic microorganisms gets access to the teat. The higher prevalence of the disease in Kafta Humera can be explained by the fact that the very hot environment in this area can interfere with the immune defense mechanism of the host which increases the susceptibility of the animals.

In the present study, age, parity, and stage of lactation didn't show statistically significant association with the occurrence of subclinical mastitis. However, Beheshti *et al.* (2010) in Iran reported a significant ($P < 0.05$) relationship with age and prevalence of subclinical mastitis where there was higher rate of occurrence in old and multiparous ewes than young and primiparous ewes. The absence of statistically significant variation with the occurrence of the diseases and these risk factors in these study sites could be explained by the husbandry factors where all animals are reared together and hence equal risks for exposure.

The present study showed for the significant prevalence of the disease which has a significant impact in the production of small ruminants. Thus, they do require good attention and management practices to control or prevent the occurrence of the disease. The proper isolation and identification of the causative organism plays a significant role in the prevention and control of the disease. Further and detailed epidemiological studies should be conducted to determine the prevalence of the disease at regional and national levels. Additionally, studies on antibiotic susceptibility tests should be done to determine the effective drug that can be used for successful treatment of the disease.

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Conflict of interest

Authors declare that they have no conflict of interest.

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