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Incidence rate of *Staphylococcus aureus* and *Streptococcus agalactiae* in subclinical mastitis at smallholder dairy cattle farms in Hawassa, Ethiopia

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A longitudinal study was undertaken from December 2009 to February 2010 at Hawassa town, Ethiopia, in smallholder dairy farms to identify the role of *Staphylococcus aureus* and *Streptococcus agalactiae* in causation of subclinical mastitis and also to assess the role of selected risk factors in the transmission of these pathogens. A total of seven farms were selected. The farms were first screened for subclinical mastitis by California Mastitis Test (CMT) and those free of the disease were monitored. Each farm was visited at intervals of two weeks and during each visit CMT was conducted. A milk sample was aseptically collected from quarters that were CMT positive (a CMT score of greater than or equal to one). Milk samples were cultured, and *S. aureus* and *S. agalactiae* were isolated. A cow found positive in CMT in the first test and had *S. aureus* and *S. agalactiae* was then excluded and was not subsequently tested and sampled. The average subclinical mastitis incidence rate due to *S. aureus* and *S. agalactiae* was found to be 21.84 ± 0.06 Sd per 100 cow- months at risk. Out of 165 CMT positive milk samples cultured for isolation of *S. aureus* and *S. agalactiae,* 88 (53.32%) yielded *S. aureus* and 30 (18.17%) had *S. agalactiae.* Co-infection by *S. aureus* and *S. agalactiae* was found in 14 (8.48%) of CMT positive milk samples. Generally, 104 CMT positive milk samples (63.03%) were due to *S. aureus* and *S. agalactiae.* Out of the 12 questions to the milking practice and other contagious mastitis control measures, only two were practiced by all farms: milking mastitic cows last and treating all cases of clinical mastitis. This study reveals that *S. aureus* and *S. agalactiae* were the major causes of subclinical mastitis and mastitis control strategies in those farms, and possibly other local dairies which have to focus on these pathogens.

Key words: Contagious mastitis, Hawassa, Subclinical mastitis**,** *Staphylococcus aureus, Streptococcus agalactiae.*

INTRODUCTION

In most Western countries it is now possible to reduce the incidence of subclincal mastitis using udder health monitoring programs by setting a regulatory limit for bulk milk somatic cell count (BMSCC) (Barkema et al., 1998). Most smallholder dairy farmers in Africa including Ethiopia are generally aware of clinical mastitis (Almaw et al., 2008; Kivaria et al., 2006) because of the signs exhibited by the cow. However, farmers' awareness of

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subclinical mastitis is very low. According to Almaw et al. (2008) none of the interviewed Ethiopian farmers knew and screened their cows for subclinical mastitis except seeking veterinarian assistance whenever their cows got sick. Generally, smallholder dairy production in North-western Ethiopia is characterized by hand milking and poor sanitary milking practices (Tassew and Seifu, 2009). This practice could facilitate the spread of contagious mastitis pathogens. Several studies have been conducted in Ethiopia and elsewhere to isolate pathogens from subclinical mastitis (Getahun et al., 2008; GIanneechini et al., 2002; Moret-Stalder et al., 2009). Contagious mastitis tends to be sub-clinical in nature. The focus on mastitis prevention and control programs has to differ between regions and should be farm specific based on the existing situations. The new infection trend of *S. aureus* and *S. agalactiae* in the absence of adequate control measure has not been studied in Ethiopia. The implementation of effective specific control program could result in the eradication of *S. agalactiae* and substantial reduction in the incidence of *S. aureus* subclinical mastitis. This study was undertaken to estimate the incidence rate of *S. aureus* and *S. agalactiae* in subclinical mastitis under the smallholder dairy production system where milking is almost always by hand.

MATERIALS AND METHODS

Farm selection

The study was conducted in Hawassa town, the capital city of Southern Nations Nationalities and People Regional State of Ethiopia from December 2009 to February 2010. A total of seven smallholder dairy farms were selected; one government owned and six private. The farms were coded as A, B, C, D, E, F and G dairy farms. The herd size ranged from 5 to 19 lactating dairy cows.

Study animals

All animals included in the study were cows producing milk at various stages of lactation. The majority were crossbreds (Holstein-Zebu) of different blood level. Only two farms had pure local breeds (Zebu). Farm D had one and F had five local zebu breeds. All the cows were hand milked and milked two times a day in the morning and evening. The animals were kept indoor, the whole day and fed roughage *ad-libtum* with nug cake supplement. Milk yield was between 4 to 20 L per day in high grade exotic breeds and 1.5 to 5 L per day in both local and low grade cross breeds.

Study design and sampling

A longitudinal study was conducted to determine the incidence of subclinical bovine mastitis due to *S. aureus* and *S. agalactiae*. The farms were first screened for subclinical mastitis using California Mastitis test (CMT). The CMT was scored 0, T, 1, 2, and 3 and was interpreted as negative, trace, weak positive, distinct positive and strong positive in that order. For the purpose of this study a score of greater than or equal to one was taken as positive. CMT was conducted according to Quinn et al. (1999). From CMT positive quarters milk sample was collected aseptically according to the National Mastitis Council (NMC, 1990) for isolation of *S. aureus* and *S. agalactiae.* Those free of the disease were monitored at intervals of two weeks for a total of six visits. This was based on the assumption that subclinically infected cows remain so at least for two weeks so that there will not be missing of cows that became sick and recovered during the interval period. A cow with a positive CMT test and had *S. aureus* or *S. agalactiae* was excluded in the next visit and was not tested and sampled for the second time to avoid persistent infection. However, cows with CMT positive but culture negative were reexamined for both CMT and bacteriology until positive for *S. aureus* or *S. agalactiae*. A cow found positive for *S. aureus or S. agalactiae* was considered at risk for *S. agalactiae* or vice versa and was monitored until found *S. agalactiae* or *S. aureus* positive or end of the study period.

Data collection

Factors that were thought to have potential association with contagious mastitis pathogens (*S. aureus* and *S. agalactiae*) and udder health problems were recorded. Twelve check lists were prepared to collect data on milking practice and other contagious mastitis control measures. The check list itmes were hand wash before milking, udder wash before milking, towel usage, milking mastitic cows last, dry cow therapy, pre and post milking teat dipping and culling of chronic mastitic cows.

California mastitis test

The California Mastitis Test was carried out as screening test to detect subclinical mastitis. 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 reagent was added to each cup. A gentle circular motion was applied to the mixtures in horizontal plane for 5 s and then the reaction was interpreted as described in study and sampling part of the paper.

Milk sample collection

Milk sample collection and storage was carried out following procedures recommended by NMC (1990) and Quinn et al. (1999). Quarter milk was collected from CMT positive cows only. The teat orifice was cleaned using cotton soaked in 70% ethyl alcohol and 5 to 10 ml of milk was collected in to sterile test tubes for bacteriological examination. During collection the test tube was held nearly horizontal to prevent contamination by dirt droppings. The sample was transported immediately to Hawassa University, Faculty of Veterinary Medicine, Microbiology Laboratory using ice box. Samples were processed immediately without storage.

Staphylococcus aureus and *Streptococcus agalactiae* isolation

Isolation of *S. aureus* and *S. agalactiae* from CMT positive milk samples was performed following standard procedures described by NMC (1990) and Quinn et al. (1999). One loopful from each milk sample was inoculated on to blood agar base enriched with 7% sheep blood. Blood agar plates were incubated at 37°C for 24 to 48 h. Each plate was examined for growth, morphology and hemolytic characteristics, Gram stain reaction and catalase tests. *Staphylococci* were identified based on catalase test, growth

Table 1. Incidence rate of subclinical bovine mastitis at cow level in smallholder farms at Hawassa, Ethiopia from December 2009 to February 2010.

Farm	Number of cows attended	Total number of cases based on culture[a]	Period of observation (month)	Contribution to cow-month at risk	Incidence rate per 100 cow-months risk
A	19	13	3	43	30
B	9	3	3	25.5	11.8
C	15	8	3	41.5	19.5
D	6	3	3	16.5	18.2
E	6	3	3	16.5	18.2
F	8	5	3	19.5	25.6
G	6	4	3	13.5	29.6
Total	69	39		176	21.84 ± 0.06sd[b] (average)

[a]*S. aureus* and/or *S. agalactiae;* [b]*sd:* standard deviation.

characteristics on Manitol salt agar and coagulase test. Coagulase positive *Staphylococcus* species (that is, *S. aureus, S. intermedius* and *S. hyicus*) were identified on the basis of acetoin production from glucose (Voges Proskauer test). *Staphylococcus aureus* is acetoin positive where as *S. intermidius* and *S. hycus* do not produce acetoin. In addition *S. hyicus* does not produce haemolysis on sheep blood agar. Isolates presumptively identified as *Streptococci* were characterized according to CAMP reaction, catalase test, and hydrolysis of esculin. A CAMP test positive *S.agalactiae* was differentiated from *S. uberis* which is also CAMP positive by production of dark brown colony on esculin blood agar (Edwards's medium) indicating esculin hydrolysis.

Data analysis

Chi square analysis was used to compare the incidence rate of subclincal mastitis between farms. The incidence rate (IR) of subclinical mastitis due to *S. aureus* and *S. agalactiae* (combined) at cow level was calculated according to the formula given in Thrusfield (2005).

$$IR = \frac{\text{Number of new cases of disease that occur in a population during a particular period of time}}{\text{The sum of overall individuals of the length of time at risk of developing disease}}$$

RESULTS

Incidence rate of subclinical mastitis caused by *Staphylococcus aureus* and *Streptococcus agalactiae*

In seven farms, a total of 69 lactating cows were monitored for the period of three months for the incidence rate of subclinical mastitis. At cow level out of 69 lactating cows 39 had subclinical mastitis caused by *S. aureus* and *S. agalactiae*. The average incidence rate was 21.84 ± 0.06 Sd per 100 cow-months. The highest subclinical incidence rate was observed on farm A (30 per 100 cow-months at risk) and the lowest was at farm B (11.8 per 100 cow-months at risk) (Table 1) and the difference was statistically significant (χ^2 (1) = 7.4667 Pr = 0.006).

Regarding the infection rate of *S. aureus* and *S. agalactiae,* there was no a continuous increase or decrease during visit period (Table 2). *S. aureus* was more prevalent than *S. agalactiae.* In farm A new infection by *S. agalactiae* ranging from 6.7 to 23% throughout all checking times was seen. And in all farms except D (*S. agalactiae* positive in the final check up) and E, which were positive for *S. agalactiae* in the previous check up, new infection by *S. agalactiae,* was seen at least in the next check up.

Bacteriology

A total of 165 CMT positive milk samples were cultured for isolation of *S. aureus* and *S. agalactiae.* Of these 88 (53.32%) yield *S .aureus* and 30 (18.17%) *S. agalactiae* (Table 3). Growth due to other bacteria was observed in 46 (27.90%) CMT positive samples but these were not further isolated. Out of 165 CMT positive milk samples, 104 (63.03%) were due to the contagious pathogens of *S. aureus* and *S. agalactiae.* Co-infection by *S. aureus* and *S. agalactiae* was seen in 14 (8.48%) samples (Table 3).

Milking practice and other contagious mastitis control measures

Of the 12 check lists which are considered important in contagious mastitis control, most of them were not in use by the farms studied (Table 4). All dairy workers used tap water to clean milking equipment, wash their hands and cows udder. All farms practiced hand milking where none of the milkers in all farms use soap to wash their hands (Table 4). The association of these milking practices with the occurrence of *S. aureus* and *S. agalactiae* were not tested statistically.

DISCUSSION

This study reveals that the majority of subclinical cases

Table 2 . Percentage of newly infected cows with *S. aureus* or *S. agalactiae* pathogens at the time of herd check-up.

Farm	Farm check up											
	1		2		3		4		5		6	
	S. aureus	*S. agalactiae*	*S. aureus*	*S. agalactiae*	*S. aureus*	*S. agalactiae*	*S. aureus*	*S. agalactiae*	*S. aureus*	*S. agalactiae*	*S. aureus*	*S. agalactiae*
A	5.3	10.5	16.7	11.8	0	6.7	13.3	7.1	0	23	15.4	10
B	11.1	0	0	0	0	0	0	11.1	0	12.5	12.5	0
C	13.3	0	0	0	7.7	0	8.3	0	11.1	6.7	25	14.3
D	0	0	0	0	0	0	16.7	0	20	0	25	16.7
E	0	0	0	0	0	0	16.7	0	0	16.7	16.7	0
F	0	12.5	12.5	28.6	14.3	0	0	0	16.7	20	0	25
G	16.7	0	20	16.7	0	20	0	25	0	0	25	0

[a]*S. aureus.*

Table 3. Frequency of *S. aureus* and *S. agalactiae* isolates in CMT positive sample in subclinical bovine mastitis in smallholder dairy farms at Hawassa, Ethiopia.

Isolate	Number	Percentage (%)
S. aureus	74	44.84
S. agalactiae	16	9.70
S. aureus and *S. agalactiae* (mixed infection)	14	8.48
Other bacateia (not identified)	46	27.90
No growth	15	9.09
Total	165	100

Table 4. Milking practices and other contagious mastitis control measures in selected farms at Hawassa, Ethiopia.

Number	Risk factor	Number of farms practicing	Percentage (%)	Farm
1	Hand wash before milking	2	28.57	A, B
2	Hand wash with soap	0	0	
3	Disinfect hand after washing	0	0	
4	Udder wash before milking	2	28.57	A, B
5	Use of towel for teat drying	1	14.28	B
6	Use of individual towel	0	0	
7	Pre-milking teat dipping	0	0	
8	Post milking teat dipping	1	14.28	B
9	Mastitic cow milked last	7	100	All
10	Dry cow therapy	0	0	
11	Culling of chronic mastitic cows	1	14.28	A
12	Treat clinical cases	7	100	All

(63.02% of CMT positive samples) were due to contagious pathogens (*S. aureus* and *S. agalactiae*) and the dominant pathogen was *S. aureus* (53.32%). This might be related to poor milking and contagious mastitis control practice seen in the studied farms. In the absence of hygienic milking practice contagious mastitis pathogens either from infected cow or milkers hand can easily spread. In cross-sectional studies of subclinical mastitis various isolation rates of *S. aureus* have been reported (Abera et al., 2010; Gianneechini et al., 2002; Moret-stalder et al., 2009). However, studies indicating the transmission rates are not common. In Germany, Sommerhäuser and his colleagues (2003) evaluated the spread of *S. aureus* in a six herds after implementing six

point control measure including strip cup testing, udder cleaning before milking using individual paper towel, post-milking teat disinfection, proper milking technique, culling and dry cow therapy. At the beginning of their study the intramammary infection rate for *S. aureus* was 24.2 to 27.1% in three herds and 4.2 to 11.9% in the other three herds. At the end of the study (Sommerhäuser et al., 2003) there was no new infection and persistent infection was observed only in one herd (1.2% of the cows) suggesting the control measures were effectively controlling the transmission of contagious mastitis. However, their study also indicated that there was dynamicity in the occurrence of *S. aureus* infection as herds which were negative in the previous check up showed 9.1% *S. aureus* new infection in the subsequent check up, in agreement with the present study. Unlike the German study, in the present study there was not a control measure in place except treating clinical cases and culling and hence the rate of new infection was bound to increase. The chance of a cow getting new *S. aureus* and *S. agalactiae* infection in a month per 100 lactating cows was found to be 21.84%. In Tanzania intervention trials were studied for a period of one year in smallholder dairy farming involving a total of 160 smallholder dairy farms with 247 lactating cows (Karimuribo et al., 2006). These studies were aimed to evaluate the effectiveness of two mastitis control practices: a single antibiotic infusion during lactation, and hypochlorite post-milking teat dipping. The result was intramammary antibiotics significantly reduced the proportion of bacteriologically positive quarters in the short-term (14 days post-infusion) but teat dipping had no detectable effect on bacteriological infection and CMT positive quarters. In the present study 30 of 165 CMT positive milk samples were having *S. agalactiae*. *S. agalactiae* an obligate parasite of the bovine mammary gland and which is susceptible to treatment with a variety of antibiotics and can be eradicated from a herd. Keefe (1997) in his review on *S. agalactiae* concluded that protocols for therapy of all infected animals in a herd were generally successful in eradicating the pathogen from the herd, especially if they are followed up with good udder hygiene techniques.

In conclusion the major causes of sub-clinical mastitis in smallholder dairy farms at Hawassa, Ethiopia were *S. aureus* and *S. agalactiae*; *S. aureus* being the dominant pathogen. This may be related to the absence of a lack of control measures for contagious mastitis pathogens observed in this study.

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