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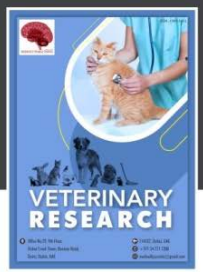

Isolation of Pathogenic Bacteria Causing Mastitis from Subclinically Infected Crossbreed Dairy Cows in Sebeta Town, Central Ethiopia

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Abstract: The flourishing markets oriented dairy farms in urban setups in Ethiopia are constrained by mastitis. In Ethiopia, a number of studies have shown a range of bacterial pathogens causing mastitis as dominant pathogenic species. This survey was designed to estimate the prevalence and identify the major bacterial pathogens involved in sub clinical mastitis in and around Sebeta area. One hundred thirty crossbred lactating dairy cows were sampled in 10 intensive farms. Following clinical assessment of the udder and teat, milk screening was done using Californian Mastitis Test (CMT). Consequently, bacteriological examination was conducted for identification and characterization of pathogens involved. The study revealed that out of the 520 quarters examined, 16 of them were blind (3.2%) and 504 were functional. From a total of 504 quarters tested for subclinical mastitis 387 (76.8%) were positive. The animal level prevalence of subclinical mastitis was 90.8% (N = 118/130). The bacterial isolates identified from CMT positive sub-clinically affected udder includes *Staphylococcus aureus* (27.2%), *Staphylococcus epidermidis* (19.1%), *Staphylococcus intermedius* (16.9%), *Staphylococcus hyicus* (2.9%), *Streptococcus agalactiae* (11.8%), *Streptococcus dysagalactiae* (5.9%), *Corynebacterium bovis* (5.9%), *Actinomyces pyogenes* (5.88%), *Streptococcus uberis* (3.7%) and *Micrococcus* species (0.8%). The observed high prevalence along with multiple potential pathogens identified is a clear indication how serious the problem is both in the studied farms and probably beyond. Thus, researchers would like to emphasize the need for urgent intervention to control the diseases and prevent the associated loss.

INTRODUCTION

Market oriented dairy farms are flourishing in and around urban setups in Ethiopia driven by the ever increasing urban population and growing demand for dairy product. However, the prevailing weak management practices, low input level and prevalence of infectious and non-infectious diseases coupled with substandard animal health service delivery are constraining the farms resulting in low productivity and profitability[1]. According to the available evidence, among infectious diseases, mastitis particularly the sub-clinical one is very much noted to contribute in affecting productivity and profitability of intensive dairy farms in Ethiopia[2, 3].

The economic lose due to sub-clinical mastitis is enormous in that it causes deterioration of the quality of milk, high cost of treatment, culling of animals at early age and food safety concerns[4]. Dairy farms suffer a huge loss before they realize the presence of the disease in the farm and take any action[5].

In Ethiopia, a number of studies have shown the occurrence of sub-clinical mastitis and a range of bacterial pathogens including S*taphylococcus*, *Streptococcus* and *E. coli* as dominant pathogenic species[6, 7]. *Corynebacterium*, *Klebsiella* and other bacteria species also reported to cause the diseases in different part of the country[8-11]. Mastitis have been indicated as a common problem in dairy farms in Ethiopia by several authors[12]. Nevertheless, reports focusing on sub-clinical mastitis prevalence and associated causative agents are limited in the study area. Therefore, this study was aimed at isolating bacterial pathogens that cause sub-clinical mastitis in dairy cows around Sebeta, one of the areas in the central highland known for market-oriented dairy.

MATERIALS AND METHODS

Study area: Sebeta is an urban set up located on the southwestern cost of Addis Ababa located 08°9200 North and 38°6200 East. The mean annual rainfall and temperature of the town are 1073 milliliters and 17.40°C, respectively. The altitude ranges from 2356-2405 m above sea level. The conurbation was purposely selected mainly due to presence of many commercial and semi-commercial farms.

Study animals and sample collection: The study was carried out on 130 lactating crossbreed dairy cows kept under intensive farming system in the study area. All ten dairy farms located in Sebeta Town were included in the study. Then simple random sampling was employed to select individual study cows. Two to twenty four lactating individual cows were selected from each farm depending on the availability of lactating cows in the farms. The study involved physical examination of sampled cows and collection of milk samples following standard procedures. The samples from each functional udder of cows were screened for sub-clinical mastitis using the California Mastitis Test (CMT, Immucell Corporation, Portland, USA).

Milk sample collection and transportation: During milk sample collection, cows were restrained in standing position and sampling began with teat cleaning by scrubbing thoroughly using cotton balls moistened with 70% alcohol. Milk samples were collected first from the closest teats followed by those at the far side of the udder by maintaining universal bottle at approximately 45° angles. The fore strip milk was discarded and 15 mL of midstream milk sample was taken. The universal bottles were labeled for information such as date of collection, name of farm and cow identification number[13]. Milk Samples were transported using cold chain from dairy farms to National Animal Health Diagnostic and Investigation Center located in Sebeta Town.

Sample storage and processing: Most mastitis causing microorganisms survive under refrigeration for several days or freezing for several weeks. The milk samples were stored in the laboratory at +4°C until laboratory examination is conducted. CMT positive milk samples were inoculated on media and incubated for 24-48 h under 37°C.

Analysis of specified sample

California mastitis test: Milk samples were collected from individual quarters into mastitis screening paddle wells, ensuring that the first strips were discarded. The results were classified as either negative or positive depending on the intensity of the reaction which is showed by gel formation. Samples with a CMT score of 0 or Trace were considered as negative while those with CMT scores of 1, 2 and 3 were considered as positive[14].

Bacteriological examination: Each milk sample collected from CMT positive udders was mixed well and a loop full approximately 0.01 mL of sub-sample was inoculated on the surface of 7% sheep blood agar and Mac Conkey plates. Inoculated plates were incubated aerobically at 37°C and examined for growth at 24-48 h[15].

The isolated microorganisms were analyzed by their colony characteristics. From culture positive plates, typical colonies were subjected to Gram's stain to see the staining properties and cellular morphology of the bacteria. Pure cultures of a single colony from the blood agar were transferred into nutrient agar plate for further biochemical examinations.

Biochemical tests: Standard biochemical methods such as catalase, oxidase, glucose, Oxidation-Fermentation

(O-F) and motility tests were conducted as primary tests. From this, a series of biochemical tests such as manitol, maltose, trehalose, Edward's medium, CAMP test, sorbitol, raffinose, salicine, aesculin hydrolysis, coagulase and other tests were conducted for final identification of various bacteria following standard methods[16, 17].

Statistical analysis: As the study was descriptive, the statistical analysis was exclusively descriptive and limited to point and interval estimates along with frequency summary.

RESULTS AND DISCUSSION

Prevalence (CMT based estimate): The results revealed that from 130 cows examined, 118 cows (90.8%) had at least one-quarter reacting positive to CMT. Of the 520 quarters 16 of them were blind (3.2%) and 504 were functional. From a total of 504 quarters testes for subclinical mastitis 387 (76.8%) were positive (Table 1). All the herds included in this study were positive for subclinical mastitis.

Table 1: Prevalence of mastitis at quarter level in selected dairy farms of Sebeta Town

Farm No.	Blind quarter	Negative quarter	Positive quarter	Total examined	Prevalence (%)
1	1	7	28	36	80.0
2	0	6	02	8	25.0
3	0	12	36	48	75.0
4	3	23	50	76	68.5
5	6	5	41	52	89.1
6	0	4	20	24	83.3
7	3	8	65	76	89.0
8	0	32	64	96	66.7
9	2	18	68	88	79.1
10	1	2	13	16	86.7
Total	16	117	387	520	76.8

Bacteriological findings: The results of the bacteriological study showed that from 118 pooled milk samples, 115 (97.5%) showed bacterial growth of 136 pure isolates. The most frequently isolated bacteria were *Staphylococcus aureus* (27.20%), *Staphylococcus epidermidis* (19.12%), *Staphylococcus intermedius* (16.90%), *Staphylococcus hyicus* (2.94%), *Streptococcus agalactiae* (11.78%), *Streptococcus dysagalactiae* (5.88%), *Streptococcus uberis* (3.68%), *Corynebacterium bovis* (5.88%), *Actinomyces pyogenes* (5.88%) and *Micrococcus* species (0.74%) (Fig. 1).

The study was carried out to isolate bacteria casing subclinical mastitis and estimate the prevalence of subclinical bovine mastitis in Sebeta Town, central Ethiopia. Overall, the disease showed a very high prevalence, i.e., 90.8% in cows and 74.42% in quarters. This study indicates that subclinical mastitis is the major dairy herd problem in the study area. This finding disagree with earlier report made by previous researchers[18] who reported lower prevalence of sub-clinical mastitis (36.67%) in the same town, Sebeta. Perhaps the lack of strategic intervention could explain such an alarming increase in prevalence during the last decade.

The prevalence of sub-clinical mastitis in this study was also higher than other previous reports[19] in Bahir Dar city (40%)[20], in southern Ethiopia (23%)[21], in Holeta town (34.8%) and[22] in Repi and DebreZeit dairy farms (38.2%). The variation in overall prevalence may be due to the effects of environment, agent and host factors involved in the causation of mastitis.

Microbiological analysis of the sample revealed that the predominant bacteria isolated was *Staphylococcus aureus* that accounted for 27.20%. This finding was in consistent with other reports[23] that indicated *Staphylococcus aureus* as predominant isolate from bovine mastitis.

The predominance of *Staphylococcus aureus* could be attributed to the wide ecological distribution of the organism on intra-mammary and skin of the udder and frequent colonization of eroded and injured skin on the teat and/or udder of the cows. This organism is well adapted to survive in the udder and usually establish mild infection of long duration and it shed through milk facilitating transmission to healthy animals mainly during unhygienic milking procedures[24, 25].

In the genus *Staphylococcus*, *S. epidermidis* and *S. intermedius* accounted for isolation rate of 16.90% and 19.12%, respectively. Mahmmoud and Shamoon[26] reported 18.18% isolation rate of *S. intermedius* in Mosul City. These pathogens are commonly described as possessing a greater ability than other common mastitis pathogens to survive and multiply in extra mammary sites and hence there is no uniform method of control proven effective against these pathogens under experimental conditions[27].

The isolation rate of *Corynebacterium bovis* (5.88%) is in agreement with the report of Duguma *et al*.[28] in Holeta Town (6.57%). Dieser *et al*.[29] reported *Corynebacterium* isolation rate of 5.2% in Argentinean dairy herds.

The isolation rate of *Streptococcus* species was 21.24% which is in agreement with the value reported by Bedane *et al*.[30] (22.5%) from Yabelo district and Asmare and Kassa[31] reported 20.3% from Wolaita zone. *Streptococcus agalactiae* isolation rate was 11.76% which is in line with that of Sylejmani *et al*.[32] who reported 14.3% *S. agalactiae* isolation rate from Kosovo. *Streptococcus dysagalatiae* accounted for 5.88% of the isolated pathogens while *Streptococcus uberis* accounted for 3.68% in this study. Harjanti *et al*.[33] reported 6.7% of *Streptococcus dysagalatiae* from Central Java, Indonesia. Michael *et al*.[34] reported 5.2% isolation rate of

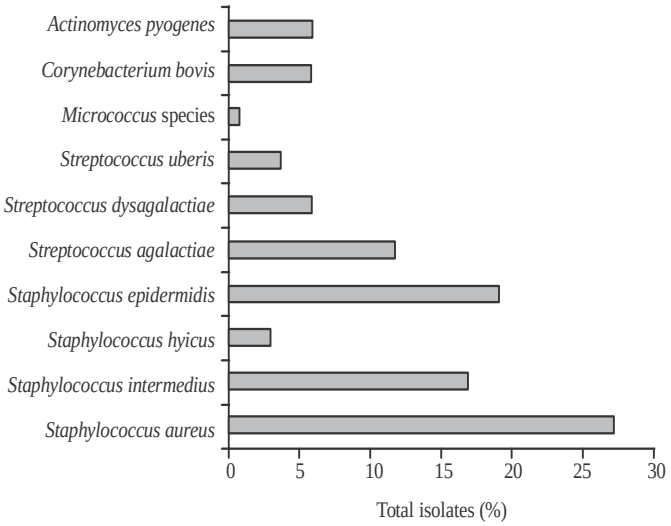

Fig. 1: Types and proportions of bacterial isolate from milk samples of dairy cows with subclinical mastitis in Sebeta Town

Streptococcus uberis in Southern Ethiopia which is very close to our finding. *Micrococcus* species accounted for 0.74% which is lower than the reports of Abera *et al*.[35] (7.14%) in Asela government dairy farm.

Actinomyces pyogenes accounted for 5.88% of the isolated pathogens which is slightly higher than the report of Al-Tarazi *et al*.[36] (4.90%) in Northern Jordan. Our finding is by far higher than that of Waage *et al*.[37] (0.5%) in Norway. This might be associated with the differences in management of dairy farms.

CONCLUSION

In conclusion, this study showed that the prevalence of subclinical mastitis is very high. *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus intermedius, Streptococcus uberis* and *Streptococcus dysagalactiae* were the most frequent isolates from milk samples collected from cows with subclinical mastitis. In general, there are clear indications for the presence of both contagious and environmental mastitis pathogens in the area that demand for special control strategy including dry cow therapy, farm hygiene improvement, regular screening and treatment and meticulous antimicrobial resistance surveillance. Thus, the animal health sector in the area and farm owners needs to be made aware of this fact for necessary measures.

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