

**PREVALENCE OF COAGULASE NEGATIVE STAPHYLOCOCCUS IN MASTITIS
INFECTION IN DAIRY CATTLE IN AND AROUND BAHIR DAR****Dr. Gashaw Getaneh***

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ABSTRACT

In Ethiopia, knowledge about the coagulase negative staphylococci (CNS) involved in mastitis is limited. Coagulase negative staphylococci have emerged to be pathogens causing intra-mammary infections in dairy herds. A cross sectional study was conducted in order to determine prevalence of CNS from October, 2014 to November, 2015 in and around Bahir Dar town. A total of 384 (129 local zebu and 255 Holstein x local zebu) lactating cows were screened. From the total lactating cows examined, 242 (63.02%) cows were found to be affected with mastitis infection which was detected by clinical examination and the California Mastitis Test (CMT), of which 14 (3.65%) and 228 (59.38%) were clinical and sub clinical mastitis, respectively. Positive milk samples were used for bacteriological examination and a total of 84 CNS were obtained. Among the potential risk factors considered, stage of lactation, parity, udder/ teat lesion, udder and teat washing before milking and after milking and breed were found to affect the occurrence of CNS mastitis significantly ($p < 0.05$). In this study, it is observed that CNS should be given a great concern as a threat for the dairy industries.

KEYWORDS: Bahir Dar, California Mastitis Test, Coagulase Negative Staphylococcus, Sub clinical mastitis.**1. INTRODUCTION**

Ethiopia holds large potential for dairy development due to its large cattle population and favorable climate for improved high yielding animal breeds. Thus, the contributions of the dairy sector especially the smallholder system in Ethiopia to poverty alleviation and sustainable food production in the country is assumed to be considerable, given the considerable potential for income and employment generation from high value dairy products. However, among many factors the sector is constrained by mastitis, which incurs serious economic losses to the dairy industry (Radostitis *et al.*, 2000).

Mastitis is an inflammation of the udder caused by a variety of micro-organisms, mostly bacteria, that gain access to the interior of the mammary glands through the teat canal. The micro-organisms live on the cow, its udder and in its environment including the floor, faeces, soil, feedstuffs, water, plant material, and milking equipment and utensils. Response to bacterial invasion and multiplication, leukocytes move from the blood stream into milk in order to fight the infection. This constitutes the inflammatory response, which may go unnoticed in the form of subclinical mastitis, or it may be severe enough to be classified as clinical mastitis characterised by physical, chemical, and usually bacteriological changes in the milk and by pathological changes in the mammary tissue. If the infection is not

contained by leukocytes or cleared through treatment, chronic mastitis may result. Such an infected quarter may lose up to 25 percent(%) of milk production and produce only poor quality milk as long as the infection still exists. Mastitis is the most common infectious diseases in dairy animals in the world which affects the dairy industry (Radostitis *et al.*, 2007).

In Ethiopia, the available information indicated that bovine mastitis is one of the most frequently encountered diseases of dairy cows. According to Lemma *et al.* (2001) of the major diseases of crossbred cows in Addis Ababa milk shed, clinical mastitis was the second most frequent disease next to reproductive diseases. Generally, the prevalence of clinical and subclinical mastitis in different parts of Ethiopia range from 1.2 to 21.5% and 19 to 46.6%, respectively (Hussein *et al.*, 1997; Bishi, 1998; Kassa *et al.*, 1999; Lemma *et al.*, 2001; Mungube, 2001; Workineh *et al.*, 2002; Kerro and Tareke, 2003).

In this study as well as in other studies, overwhelming cases of mastitis were subclinical compared to clinical mastitis in both breeds (Kassa *et al.*, 1997; Hussein, 1999; Workineh *et al.*, 2002; Kerro and Tareke, 2003; Enyew, 2004). According to Radostitis *et al.* (1994), an infected quarter showed 30% and a cow 15% reduction in milk yield. Usually Ethiopian farmers especially smallholders are not well informed about the invisible

loss from subclinical mastitis (Hussein, 1999) since dairying is mostly a sideline business in these farmers. Among a wide variety of mastitis causing microorganisms, bacteria are the most frequent pathogens of these diseases. Although variation exists on the type and isolation rate from country to country, the most commonly incriminated in intramammary infection (IMI) are *Staphylococcus* species, *Pasteurella* species *Streptococcus agalactiae*, *Escherichia coli* and *Bacillus* species (Jubb and Kennedy, 1997). To date, more than 50 *Staphylococcus* species and sub-species have been characterized to cause staphylococcal mastitis. In mastitis diagnosis, staphylococci are divided into coagulase positive and coagulase negative *Staphylococcus* (CNS) on the basis of the ability to coagulate rabbit plasma. In diagnostics of bovine mastitis, the clarification has been considered adequate because CNS usually course subclinical or only mild clinical mastitis. Hence, it is consider as minor pathogen (Koivala *et al.*, 2007; Lim *et al.*, 2007). It seems that CNS mastitis is a particular problem in well managed high producing farms, which have successfully controlled udder infections caused by major mastitis pathogens (Myllys and Rautulu, 1995).

CNS have become the most common bacterial pathogens isolated from sub-clinical mastitis in many countries (Simojoki *et al.*, 2009) and could be described as emerging mastitis pathogens (Pyorala and Taponen, 2009). CNS are normally found on the healthy skin of the nipple and the hands of the milker. They are often called opportunistic microorganisms because they live in areas where it is easy to colonize the teat canal and penetrate the secretary tissue. Implementing mastitis control programs over the past 30 years has led to a reduction in the overall incidence of clinical mastitis in most herds. In some cases, the decrease has been 90%. Whereas the clinical disease caused by major pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* decreased significantly, less important pathogens such as CNS have been increasingly taking on greater importance. Cows and heifers can be infected with CNS before calving. In lactation, infection due to CNS is associated with an increase in somatic cell count (SCC), which causes economic losses due to the penalty in the price of milk. CNS are a groups of bacteria and a variety of CNS species has been isolated from mastitis. *S. chromogenes*, *S. simulans* and *S. hyicus* are reported most often, but also many other species are frequently mentioned.

In Ethiopia, knowledge about the coagulase negative staphylococci (CNS) involved in mastitis is limited. Coagulase negative staphylococci have emerged to be pathogens causing intra-mammary infections in Ethiopian dairy herds. Particularly in Bahir Dar there is no recent study on the importance of coagulase negative staphylococcus in mastitis infections in which owners only concentrate on the treatment of clinical cases and thus sub-clinical cases are neglected. Therefore, the

present investigation was undertaken: To study the prevalence of coagulase negative Staphylococcal causes of bovine mastitis.

MATERIALS AND METHODS

Study area

The study was conducted in and around Bahir Dar town between October, 2014 to November, 2015. Bahir Dar is the capital city of Amhara region and is located at about 563 km north-northwest of Addis Ababa, having a latitude and longitude of 11°36'N 37°23'E coordinates respectively with an elevation of about 1,800 meters (5,906 feet) above sea level. The area covers 28 km² and receives high rainfall from May to October and low rainfall from November to March with maximum of 1683 mm and minimum of 93.4 mm (NMABB, 2013).

Study population

The study populations were all lactating local zebu (Fogera) and Holstein-zebu cross bred cows from dairy farms in and around Bahir Dar.

Study design

Study type

Cross sectional type of study was carried out on 47 different small holder farms and Andassa Livestock Research Center through simple random sampling method from October, 2014 to November, 2015.

Sample size determination and sampling methods

The sample size was calculated according to the formula given by Thrusfield (2005). It was calculated by taking 50% estimated prevalence since there was no previous study and at 95% confidence interval and 5% precision level. Simple random sampling method was considered to select the individual dairy cow. The sample size was determined using the formula:

$$N = 1.96^2 p_{exp}(1-p_{exp})/d^2$$

Where N=required sample size, Pexp = expected prevalence d²=desire absolute precision

The sample size value was determined by substituting the given data required and found to be 384 animals required from the study population.

Study methodology

Data collection

A semi-structured questionnaire was developed and pretested, and all information relating to study objectives was recorded. Data collected includes type of housing, breed, age, parity, lactation, previous mastitis history. Udder and milk abnormalities (injuries, blindness, tick infestation and indurations swelling, milk clots abnormal secretions) were also recorded.

Detection of mastitis

Physical examination of the udder

The udder was first examined visually and then through palpation to detect possible fibrosis, cardinal signs of inflammation, visible injury, tick infestation, atrophy of

the tissue and swelling of the supra-mammary lymph nodes. Rectal temperature of those cows with clinical mastitis was taken to check systemic involvement. Information related to the previous health history of the mammary quarters and cause of blindness was obtained from case record sheets when available and by interviewing the farm owners when not. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood and watery secretions (Radostitis, 2007).

CMT screening

Sub-clinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture (milk and CMT reagent), which show the presence and severity of the infection, respectively (Harmon, 1994). Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities and were screened by the CMT according to Quinn *et al.* (1999). From each quarter of the udder, a squirt of about 2ml milk sample was placed in each of the cups on the CMT paddle and an equal amount of CMT reagent was added to each cup and mixed well in gentle circular motion for 15-20 seconds. Reactions were graded as 0 and Trace for negative, +1, +2 and +3 for positive (Quinn *et al.*, 1999).

Sample collection

Milk sample collections were done according to the procedures recommended by National Mastitis Council (NMC, 1999). The udders and especially teats were thoroughly washed with clean water and dried by towel. Then each teat ends were disinfected with cotton swabs soaked in 70% alcohol and allowed to dry. Separatepledged cotton is used for each teat. Prior to sampling, the first 2-3 streams of milk were discarded, and Teats towards sample collection sampled first and then the far ones. The collecting vial was held as near horizontal as possible, and by turning the teat to a near horizontal position, approximately 10 ml of milk was collected into a universal sample collection bottle.

Handling and storing samples

After collection, the samples were held in an ice box and transported to Bahir Dar regional micro biology laboratory. In the laboratory samples were immediately processed or stored at 4°C until culture NMC (1999).

Table 1: Total isolated staphylococci and CNS from bovine mastitis of lactating cows.

| Forms of mastitis | Staphylococcus at cow level (%) | Staphylococcus at quarter level (%) | CNS at cow level (%) | CNS isolated at quarter level (%) |
|--------------------------|--|--|-----------------------------|--|
| Clinical | 7 (1.82) | 17 (1.12) | 2 (0.52) | 3 (0.20) |
| Sub-clinical | 86 (22.40) | 180 (11.72) | 39 (10.16) | 81 (5.27) |
| Total | 93 (24.22) | 197 (12.83) | 41 (10.68) | 84 (5.47) |

Risk factors affecting prevalence of CNS

Eight factors were investigated for determining the potential risks for the occurrence of mastitis in this study. These were breed, stage of lactation, parity, Previous

Bacteriological examination

Bacterial isolation

Bacteriological examination of milk was carried out following the standard procedures (Quinn *et al.*, 1994). One standard loop (0.01ml) of milk sample was streaked on 5% blood agar. The inoculated plate then was incubated aerobically at 37°C for about 24 to 48 hours. The plate was examined for growth after 24 or 48 hours. For primary identification, colony size, shape, color, hemolytic characteristics, and Grams reaction were used. Suspected colonies were sub-cultured onto nutrient agar plate for other biochemical tests.

Data analyses

Data collected from each study animal and laboratory work results were coded in to appropriate and enter in Microsoft excel spread sheet. Then analyses were performed using Statistics Package for Social Science (SPSS) version 20. Association of specific variables breed, parity, age, stage of lactation, milk yield and udder washing were performed by using Pearson chi-square (χ^2). P-values were calculated and $P \leq 0.05$ was considered as statistically significant.

RESULTS

Prevalence of mastitis and CNS

A total of 384 lactating cows (129 local indigenous and 255 cross breeds) were examined for mastitis detection and out of which 242 (63.02%) cows were found to be affected with mastitis infection, of which 14 (3.65%) and 228 (59.38%) were clinical and sub-clinical mastitis, respectively. The appearance of milk secretion from clinical mastitis was found to be clots, flakes, bloody and watery secretions. Sub-clinical and clinical prevalence at cow level was 182 (71.37%) and 13 (5.10%) in cross breed and 46 (35.66%) and 1 (0.78%) in local zebu (Fogera) breeds, respectively. Out of the 1536 quarter examined 68 (4.43%) quarters which belongs to 57 (14.84%) animals were found to be blind teat. Up on screening of the functional teats (1428) by CMT, a quarter of 651 (45.59%) were found to be affected by sub-clinical mastitis. The total isolates of staphylococci were 38.43% from the total CMT screened positive cows and 43.97% at quarter level. Out of this 44.08% and 40.61% CNS were isolated at cow and udder quarter level, respectively (Table 1).

history of mastitis, milk yield, age, Udder/teat washing and presence of lesion on udder or teat. Prevalence of CNS within breed ($p=0.036$), parity ($p=0.00$), age ($p=0.012$), tick/teat lesion ($p=0.00$), Udder/teat washing

($p=0.0364$), milk yield ($p=0.002$) and stage of lactation ($p=0.016$) were found to be statistically significant (Table 2). For stage of lactation significant difference was observed between late and early stage of lactation ($p=0.00$) and ($p=0.016$), respectively. The occurrence of CNS recorded at both late and early lactation was 19.85% and 30%, respectively. Similarly, the isolation rate of CNS for cows gave birth 6 and above was 21.05% ($p=0.03$) and cows gave birth <3 and 4-6 were 35.2% ($p=0.00$) and 9.6%, respectively. The occurrences of CNS were significantly associated and high in cross breed (25.10%) than local breed (15.50%) ($p=0.036$).

Table 2: Risk factors associated with the occurrence of CNS cause of bovine mastitis on dairy cattle.

| Variables | Group | Number of animals | CNS Positives (%) | χ^2 | P-value |
|---------------------|---------------|-------------------|-------------------|----------|---------|
| Age | <5 years | 219 | 58 (26.48) | 6.335 | 0.012 |
| | 6-8 years | 165 | 26 (15.76) | | |
| Parity | <3 | 125 | 44 (35.2) | 21.043 | 0.00 |
| | 4-6 | 125 | 12 (9.6) | | |
| | 6-8 | 133 | 28 (21.05) | | |
| Lactation stage | 1-120 days | 131 | 39 (30.0) | 8.311 | 0.016 |
| | 121-240 days | 123 | 19 (15.45) | | |
| | >240 days | 130 | 26 (19.85) | | |
| Milk yield | Low (0.9L-4L) | 168 | 24 (14.29) | 10.066 | 0.002 |
| | High (>4L) | 216 | 60 (27.28) | | |
| Breed | Cross bred | 255 | 64 (25.10) | 4.614 | 0.036 |
| | Local zebu | 129 | 20 (15.50) | | |
| History of mastitis | No | 228 | 51 (23.37) | 0.080 | 0.803 |
| | Yes | 156 | 33 (21.15) | | |
| Udder/teat lesion | Present | 72 | 44 (61.11) | 79.826 | 0.00 |
| | absent | 312 | 40 (12.82) | | |
| Udder/teat washing | No | 259 | 67 (25.87) | 4.379 | 0.0364 |
| | Yes | 125 | 17 (13.6) | | |

χ^2 =Pearson chi-square.

P-value=probability.

DISCUSSION

In this study most of CNS were isolated from sub-clinical mastitis as compared to clinical mastitis. This is in line with Mekbib *et al.* (2010) and Mekonen *et al.* (2005) who reported the prevalence of CNS in sub clinical was 28.75% and 12.01% which were higher than CNS from clinical mastitis in which 1.33% and 3.53% in Holeta and in Addis Ababa dairy farms, respectively. In the study made by Molalegne *et al.* (2010) it is indicated that CNS were not isolated from clinical mastitis but 51.9% of CNS were isolated mainly from sub-clinical mastitis in Bahir Dar.

In this study the isolated CNS at cow level is in line with the result of Adisalem and Mersha (2012) who reported 11.93% in Addis Ababa dairy farms. The current finding also comparable to the results of 16.6% reported from Egypt by Ahmed *et al.* (2013). Reports from Sweden showed that the total isolates of CNS at udder quarter level was varied from 7.2% to 17.5% in herd Thorberg *et al.* (2000) and this is closely agreed with the current finding.

However, no significant difference between previously mastitis infected cows and non-infected cows ($p=0.803$, Table 2) were observed. Higher prevalence of CNS were isolated from age group of <5 year in which 26.46% than cows belongs to age group 6-8, where 15.76% ($p=0.012$). Prevalence of mastitis was significantly associated with udder/teat injuries that as many as 61.11% of cows with udder/teat injuries had mastitis compared with only 12.82% of cows with no injuries. High producing cows (27.28%) were found to be more susceptible than low producing cows (14.29%) ($p=0.002$).

The prevalence rate of CNS at quarter level in this study is comparable with the findings of Enyew (2004) and Hussein (1999) who reported 49.63% in Bahir Dar and 42% in Addis Ababa, respectively. However, the present finding lower than Bishi (1998) who reported that 54% in Addis Ababa and higher than reports of Belayneh *et al.* (2013) and Regassa *et al.* (2009) who reported 18.7% and 31.7% of CNS in Adama and Holeta town, respectively. Abunna *et al.* (2013); Aragaw *et al.* (2012); Kerro and Tareke (2003) and Workineh *et al.* (2002) reported the isolation of CNS at a rate of 11.27%, 21.43%, 2.5% and 30% of the total isolates, respectively in different part of the country and lower than the present study. In Poland, in a survey of mastitis in dairy herds of small type farms in the Lublin region, CNS was isolated at a higher rate (36.6%) compared to other pathogens (Krukowski *et al.*, 2000). A higher finding was also reported in India in mastitis survey by Basappa *et al.* (2011) where CNS was 58.06%.

The isolation rate of CNS mastitis varies markedly between studies as reviewed by (Taponen *et al.*, 2007). The variation may at least partly be due to differences in

sampling and diagnostic techniques, making direct comparisons between studies difficult. Other factors of importance are parity and stage of lactation. The isolation rate variability of CNS in different studies and also in this study could also be associated with lowered resistance of the cow may due to udder/ teat injury. In comparable to the current study, Biffa *et al.* (2005) and Molalegne *et al.* (2010) who reported that as 68.8% and 90.3% of cows with udder/teat injuries had mastitis compared with only 18.2% and 21.8% of cows with no injuries in Addis Ababa and in Bahir Dar, respectively.

According to several studies in Ethiopia, the highest prevalence of CNS intramammary infection occurs in heifers around parturition (Moges, 2012; Delelesses, 2010; Tesfaye, 2009; Biffa *et al.*, 2005; Alemnew *et al.*, 1999; Matthews *et al.*, 1992). In support of those studies, the present study revealed that the isolated CNS was higher in early lactation stage (than mid and late lactation stages). In agreement with this result Zeryehun *et al.* (2013) and Biffa *et al.* (2005) also reported that early stage and the period of involution of the mammary glands were the most susceptible stages with prevalence of 87.2%, 73.1% and 45.8%, 38.7%, respectively. They also noticed that the prevalence of CNS for cows at mid lactation was 65.9% and 25.8% in and around Addis Ababa and in southern Ethiopia, respectively. This could probably be associated with the ability of the immune system of an animal to defend infection causing agents. Absence of dry cow therapy regime could possibly be among the major factor contributing to high prevalence at early lactation. Isolation rate of CNS in late lactation was greater than mid lactation. Since during dry period due to the low bactericidal and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply.

In the present study, breed and milk yield were found to have effect on occurrence of bovine mastitis. Similar results was suggested by Molalegne *et al.* (2010) who reported that the prevalence of mastitis in cross breed was 36.7% and local breed was 17.7% in the study conducted in Bahir Dar. The difference in occurrence of mastitis in these breeds could arise from differences in cellular immunity (Erskine, 2001). In this study the prevalence of CNS mastitis for high milk producing cows was higher than the low producing cows. Increases in milk yield from genetic selection may be accompanied in genetic susceptibility to mastitis (Schutz, 1994). According to Grohn *et al.* (2004) high producing cows were susceptible for CNS than low milk producing cows. This is due to the reason that higher milk production has affected the capacity of the immune system of dairy cows to combat infections, and the associated bacteria have adapted to changes in their hosts and environment.

In the present study udder washing also found to have effect on the occurrence of CNS mastitis, higher in animals/farms where udder washing is not practiced. In support of this study, Radostitis *et al.* (2007) explained

that a hygienic condition plays a great role in the prevalence of CNS from bovine mastitis. Zeryehun *et al.* (2013) also reported that farms that used udder washing have prevalence of mastitis was 62.86%, while those of the farms they did not used udder washing the prevalence have been 79.67% in Addis Ababa. However, in the present study revealed that previous mastitis history was not statistically significant in the occurrence of CNS in bovine mastitis. In contrast to this, Biffa *et al.* (2005) reported 57% of cows with previous mastitis had mastitis compared with only 22% of cows with no previous mastitis in Addis Ababa. This shows animals having previous mastitis history more sensitive than non infected one and hence prevention and control of subclinical mastitis by giving great attention.

CONCLUSION AND RECOMMENDATIONS

Coagulase negative staphylococci cause of mastitis is becoming a major health problem of dairy cows in the study area and undoubtedly will have an adverse effect on productivity of dairy industry. It is recognized that multiple environmental and managemental factors plays a major role in the occurrence of this pathogen. Hence, remains as a major agent for the occurrence of sub-clinical mastitis infection in the study area, which results in the economic loss of the dairy outputs.

Based on the above facts the following recommendations are suggested:

- Mastitis infection caused by CNS warrants a serious attention
- Management and environmental factors should be regulated.

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