Bovine Mastitis: Prevalence, Risk Factors and Isolation of Streptoccocus Species from Small Holders Dairy Farms in and Around Haramaya Town, Eastern Ethiopia

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Abstract

Mastitis is the most complex and costly disease of dairy cows occurring throughout the world including Ethiopia. Streptococcal mastitis is the commonest and economically important. However, mastitis caused by this species is not well investigated. A cross-sectional study was conducted from November 2016 to April 2017 to determine the prevalence of mastitis, associated risk factor and also to isolate pathogenic streptococcus species from lactating dairy cows in and around Haramaya town, Eastern Ethiopia. A total of 384 milking cows and 1536 quarters were examined, out of which 189 and 677were CMT positive at cow and quarter level respectively. The overall prevalence 49.2

Index terms— isolation, mastitis, prevalence, streptococcus species.

1 I. Inroduction

thiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to rally round the economic development of the country. Cow represents the biggest portion of cattle population of the country (CSA 2016). Milk produced from these animals provides an important dietary source for the majority of rural as well as considerable number of the urban and per-urban population. However; milk production often does not satisfy the countries requirement (FAO, 2003).

Mastitis is the common and costly disease causing loss in milk yield, treatmentcost, milk discarded, and reduction in quality and quantity of milk produced by a cow. Bacterial contamination of milk from affected cows may render it unsuitable for human consumption by causing food poisoning or interference with manufacturing process or in rare cases, provides mechanism of spread of disease to humans. Zoonotic diseases potentially transmitted by raw cow milk include brucellosis, leptospirosis, listeriosis, Q-Fever, Staphylococcal food poisoning and tuberculosis (Radostits et al., 2007).

By definition mastitis is inflammation of mammary gland parenchyma which is caused by noninfectious agents or microorganisms usually bacteria that invade the udder, multiply and produce toxins which are harmful to the mammary gland (Erskine, 2003 **??** Mekonnen et al., 2005), is classified as clinical and sub clinical. Clinical mastitis is characterized mainly by appearances of changes in the milk such as flakes and clots and presence of signs of inflammation on the mammary glands such as swelling, heat, pain, and edema (Christos, 2011). Subclinical mastitis refers to inflammation of the mammary gland in the absence of visible gross lesion in the udder or its secretion with the presence of pathogenic microorganisms and usually high number of somatic cells in the milk **??**DACA, 2006), milk production decreases, bacteria are present in the secretion, and composition is altered (Blowy, 2010).

Majority of microorganisms that are responsible for mastitis and spoilage of milk are bacterial origin include Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli and Streptococcus uberisas dominant and pathogenic (Mungube et al., 2005). Streptococci are one among the major mastitis pathogens which have

a considerable impact on cow health, milk quality and productivity (Mungube et al., 2004). Streptococcus-agalactiae is causes contagious mastitis, an obligated pathogen of the mammary gland, which is transmitted directly among cows during milking **??**NMC, 2004). It infects the gland cistern and ducts of the mammary gland causing irritation; swelling and subclinical mastitis (Hillerton and Berry, 2003). As a result, S. agalactiae can spread widely within a herd, causing immediate loss due to reduced milk yield **??**Zoccone, 2006).

Streptoccocu. dysgalactiae is described as alpha hemolytic and associated only with IMI among the environmental streptococci; S. dysgalactiae is one of the most prevalent, which may infect mammary glands as favorable conditions arise **??**Hillarto et al., 2005). Streptoccocus. uberis is an important udder pathogen in the modern dairy industry (Pullinger et al., 2006). The severe economic impact caused by the high prevalence of environmental streptococci in well managed dairy herds (Leigh, 1999).

Mastitis is an important factor that limits dairy production due to its heavy financial losses involved and the existence of latent infections characteristics (Lasagno et al., 2011). The control and prevention of such important disease in the dairy sector require a rigorous and systematic research on the status of the disease. However, in some parts of Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant.Moreover, many investigations on bovine mastitis in Ethiopia focused on Staphylococcus aureus, Escherichia coli, and rarely streptococcus. Despite therecognition of streptococcal mastitis all over the world (Lasagno et al., 2011), the information on bovine streptococcal isolates from Ethiopia is scarce. Therefore, the objectives of this study were to estimate the prevalence of bovine mastitis, assess the risk factors and also to isolate streptococcus species from lactating dairy cows in and around Haramaya town.

2 II. Material and Method a) Study Area

The study was conducted in and around Haramaya town, such as Haramaya townAdelleWaltaha, Tuji-gabisa and Ifa-Oromiakebele at Haramaya district of Eastern Hararghe,Oromia region. Haramaya district is located in the Eastern Hararghe Zone of the Oromia Region of Ethiopia, which are about 506 kilometers from Addis Ababa and 12 kilometers far from the city of Harar and 35 kilometers from Dire Dawa and 5 kilometers from Haramaya University at an altitude of 2047 meters above sea level (m a.s.l.) between latitude 9°24??N and longitude 42°01??E. The mean annual rainfall is 870 mm with a range of 560 to 1260 mm and the mean maximum and minimum temperatures are 23.4°C and 8.25°C, respectively relative humidity of 68% (HADB 2016). Small holder mixed farming system is the dominant mode of production of the farmers in the area. The district has about 76,336 cattle, 65,083 sheep, and 84,916 goats, 22,355 donkeys, 356 camels and 89,800 chickens. The area receives an average annual rain fall of approximately 900 mm, with a bimodal distribution pattern **??**PSE, 2015).

3 b) Study Population

The study populations were lactating cows of small holder dairy farm which were breeds kept under the semi-intensively husbandry practice and there milking practice was by hand (manual). Lactatingcowsin Haramayatown, Adele Waltaha, Ifa-Oromiya and Tuji Gabisa, were the animals included in the study. These animals werekeptunder the semi-intensive management system whereby cattle are grazed freely on pasture but received supplementary feeds in the morning and evening when they were milked and during last pregnancy. All cows were hand milked twice daily, in the morning and evening. The milk yield of the cows ranged from (4-8 L) per day for cross breeds while (2-4L) for local breeds.

4 Sample Size Determination

Across sectional study was conducted to determine the prevalence of both clinical and subclinical mastitis after a total 384 cow's milk samples were collected by simple random sampling from expected prevalence is 50% CMT with the 95% confidence level and desired precision of 5% using the formula described by Thrusfield (2005). n = 1.96 2 × P exp (1-P exp) =384

5 d) Sampling Strategy

A cross-sectional study was carried out to determine bovine mastitis from November, 2016-April, 2017 conducted on simple random sampleselected local and cross breed lactating dairy cows from selected area in and around Haramaya town at cows level based on udder inspection for clinical mastitis manifestations and indirect test (California mastitis test) for sub clinical mastitis, questioner survey for risk factor and milk sample collection for microbial isolation.

6 e) Sampling Method

Sample collection was made to examine all functional teats of each study animals and CMT positive cases with relevant information about lactating cows in the small dairy farm was gathered and the sample was employed from CMT for the bacterial isolation.

7 f) Questionnaire Survey

A semi-structured questionnaire was developed and pretested, and all information relating to the study objectives was recorded. Data collected include address and Pertinent to cow-level factors, including lactation dairy cows age, parity, lactation stage, breed and milking practice where the owner of cows were wash hand and udder before and after milking, wash hand and udder before milking and wash hand only before milking. Age of the animals was determined from birth records and dentition characteristics and categorized as young (>3 to 6 years), adults (>6 to 10 years), and old (>10) according to Jonsan(1999) who classification of age depending dentition. Stage of lactation was categorized as early (1 st to 3 th month), mid (4 th to 6 th month), and late (7 th month to the beginning of dry period). Parity was categorized as few with (1-3 calves), moderate (4-6 calves) and many (7 and above calves).

8 g) Clinical Inspection of the Udder

Each cow was clinically observed for the manifestation of general clinical signs related to udder and teats and presence of any gross abnormalities. The udder was first examined visually and then through palpation to detect possible fibrosis, inflammatory swellings, visible injury, tick infestation, atrophy of the tissue, and swelling of supra-mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood, and watery secretions. The udder was also inspected for the presence of any grossly visible injury on location, size, and nature injuries the teats were part of the indicators for clinical mastitis (Quinn et al., 2002).

9 h) Milk Sample Collection, Methods of Transportation and Storage of Samples

The Californian mastitis reagent was used to screen cows with sub clinical mastitis milk sample collection was according to the procedures recommended by national mastitis council (NMC, 1999). The result of the test was indicated on the basis of gel formation. The interpretation (grades) of the CMT was evocated and the results graded as 0 for negative and trace 1, 2 and 3, for positive (Quinn et al., 2002).

The milk sample was taken from cows, washing by clean water and dry the teat by cotton and the teat were wiped thoroughly with 75% ethyl alcohol and the first stream (2-3) of milk from each quarter was discarded and collected milk in the sterile milk collection bottle for good collection of sample. After collection of the milk sample, all samples were clearly labeled with the appropriate identification of the cows, quarter using permanent marker on the test tube and all samples were transported with ice box to the laboratory without delay and it were processed (Quinn et al., 2002). In the laboratory, samples were cultured immediately or stored at +4 0 c in any case of delay **??**NMC, 2004). Analysis o f specified samples was performed on isolation and identification of pathogenic bacteria at Haramaya University collage veterinary medicine laboratory in microbiology laboratory.

10 i) Detection of sub-clinical Mastitis

Mastitis was detected using the California Mastitis Test (CMT) and results of clinical inspection of udder **??**Quinn et al., 1999). Grades of the CMT were evaluated and the results graded as 0 for negative and1, 2 and 3 for positive **??**NMC 2004). Subclinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture, which show the presence and severity of the infection, respectively (Harmon 1994)

11 j) Preparation of Culture Media, Culture and Bacterial Isolation i. Preparation of Culture Media

To prepare media for bacterial culture, the manufacturer's instructions was be followed, besides few additional general points were included, all glass wares used for the preparation of media were first sterilized using appropriate equipment like autoclave, hot air oven, the appropriate amount of dehydrated media were weighed out of using sensitive balance and the required amount of distilled water were added to the powder media. Dehydrated media containing agar were dissolved in heating mantle until it boil and frothy appearance was settled (removed), then the media were sterilized by autoclave at 121 0 C for 15 min holding time, and cooled in water bath at 50 0 C before poured in to the Petri dishes. Some media like blood agar and modified Edward medium requires addition of blood after it is cooled to 50 0 C since RBC are not tolerate higher temperature, adapted from (Quinn et al., 2002). The common media used during the study were blood agar, MacConkey agar, modified Edward medium (Oxiod England), Aesclinehydirolaysis media and Manitol salt agar.

12 ii. Culture and Bacterial Isolation

After Milk samples were collected from all quarter with clean and aseptically procedure for microbiological culture and species identification, C according to the procedures of the (NMC, 1999). Culturing of milk sample collected from individual cows, in search for mastitis producing organisms in standard of examination for mastitis (Radostits et al., 2007). One standard loop (0.01 ml) of milk sample was streaked using the quadrant streaking

method for each cows on streptococcus selective agar of modified Edward medium (Oxiod England) at around Bunsen burner to reduce contamination. In case of refrigerated milk samples, as bacteria might be concentrated in the cream layer and held with in clumps of fat globules, dispersion of fat and bacteria was accomplished by warming the samples at 25 °C for 15 min before plating on modified Edward medium agar the inoculated plates were then incubated aerobically at 37 °C for 24 to 48 hrs.

Then the inoculated plates were examined from 24hr incubation to 48hrs for growth, morphological features, such as colony size, shape, and color, and hemolytic characteristic, the growth colonies on selective media were sub-cultured on 7% sheep blood agar (Oxoid, UK) for further investigation hemolytic types and growth character. After pure colonies were obtained, Gram stained smears were done for primary identification of bacteria to genus level, such as Gram reaction (Gram positive and Gram negative), and cellular morphology (coccus or rods). Other primary tests had done include catalase, oxidase and growth or absence of growth on MacConkey agar (Oxoid, UK) and the secondary biochemical tests such as, CAMP test Aesculin hydirolaysis test, etc were done for bacterial species identification. annex 3

13 k) Data Management and Analysis

The collected data were entered to Microsoft office excel 2010 program and analyzed using SPSS version 20. Descriptive statistics were used to summarize the generated data on the rate which was collected through, clinical inspection, CMT, isolation and identification Streptococcus species. Prevalence of mastitis related to specific risk factors was determined as the proportion of affected cows out of the total examined. Effects of specific variables (breed, hygienic practice, age, parity, lactation stage, site, on prevalence of mastitis were investigated using chi-square (X 2) test. Similarly, the variation in prevalence of mastitis-induced blind quarters was assessed using the same statistical method. A statistically significant association between variables is considered to exist if the p value is < 0.05.

14 III. Results

A total 384 lactating cows were included in thisstudy and 189 (49.2%) cows were found be positive for mastitis on CMT. Out of 189 CMT positive cows, 29/384 (7.5%) clinical and160/384 (41.7%) sub-clinical mastitis were found with statically significance difference (p=0.000) table 1. b) Quarter Prevalence of Mastitis using CMT A total number of quarters (1536) of cow were checked for the presence of gross abnormalities, 54 quarters were found to be blind teats and 1482 quarter were using CMT screening test out of these 677 (45.68%) quarters were found to be positive mastitis on CMT positive at quarter levels (table 3).

15 d) Risk factors associated with bovine mastitis

During the course of study on varies risk factors associatedmastitis among those age, parity, lactation stage, breed, milking hygienic practice and address of animal for examine presence of mastitis at cow's level. The age, parity, lactation stage and milking hygienic practice were found to be significantly ($p<0.05$) associated with presence of mastitis. On another hand breed and address did not significant effect ($p>0.05$) on presence of mastitis (table 5).

There were significant differences in prevalence between cows of different age categories. The highest prevalence (66.6%) was found to be lactating cows at old age (>10 years old) and followed adult cows with age category between (6-10) years (51.6%), and the lowest prevalence (42.5%) was recorded in young cows at age category between (3-6) years old with significant at (p=0.004).

Risk factors with lactation stage between successive lactation stage were significant effect (P=0.000) on the prevalence of mastitis. Higher prevalence (64.3%) of mastitis was observed and recorded in cows of earlier lactation stage between first three months of lactation (1-3 month), followed by cows in late lactation stage (7 th month to the beginning of dry period) (52.7%) and lowest prevalence (30.5%) was recorded cows at middle lactation stage between (3 month to 6 month) (table 5).

There was also statically significant difference in prevalence between lactating cows at different parity (P=0.003). The highest prevalence (72.9 %) was recorded in cows which gave birth up to 7 and above calves, followed by cows which gave birth or parity number between 4-6 calves (51.6%) and the lower prevalence (42.9%) was recorded in cows that gave birth to 1-3 calves (table 5).

The milking hygienic practices of udder during milking were significant effect with Presence of mastitis (p=0.000). The highest were found the cows managed under poor milking hygienic practice (no udder and hand washing) (86.3%), followed the cows which wash udder and hand before milking (33.9%) and lowest prevalence (22.6%) were recorded cows at good milking hygiene practice (wash before and after milking) (table 5). The presence of mastitis with cows address was also studied;but the result on statistical analysis indicated were not significant difference ($P > 0.05$) among different kebele in the study area (table 5).

16 C

The effect of breed on the presence of bovine mastitis at study area were revealed that breed with in prevalence of subclinical and clinical mastitis did not vary along with the breed of animal, but relatively higher prevalence was

seen in animals at local breed (56.6%) and low in cross breed with prevalence of 43.9%. The result of statistical analysis revealed no significant difference (P > 0.05) among the breed animals (table 5).

17 IV. Discussion

In the current study,a total of 1536 quarters and 384 lactating cows in and around Haramaya town east Hararghe were investigated and overall prevalence of mastitis 49.2% at cows levels were recorded. This result was in agreement with (Sori et al., 2005) Benta and Habtamu (2011) in Batu and its environments, Ethiopia 5.3% on prevalence of clinical mastitis. These variations could be due to improper hygiene during udder preparation and milking, lack post milking dipping of teats and appropriate treatment. Risk factors which influence the occurrence of clinical mastitis were outlined as animal, pathogen, and environmental risk factors, which could contribute in the discrepancies of mastitis prevalence (Radostits et al., 2007).

Out of examined cows, 160/384 (41.7%) were found to be positive forsub-clinical mastitis. This result was in agreement with previous findings such as 40 Fadlelmoula et al. (2007). The difference may be due to greater experience in drying off, the potential effect of level of milking hygiene and cleanness, and the application of sanitary measures.

The study result revealed statistical significant association of prevalence of mastitis with the age, lactation stage, parity and milking hygiene practice of lactating cows. The present result was coincides with previous study that state increasing age, lactation stage, parity and poor management as the risk of mastitis (Dego and Tareke, 2003) and Nibret et al., 2011). The association of age with positivity for mastitis was found to be statistically significant (P<0.05) and high prevalence of mastitis was recorded in old cows. This finding was found to be similarl with previous finding of Girma **??**2010) in Holeta area and Bitew et al., 2010 around Bahir Dar area. The higher prevalence in older cows in the present study might be that older cows have largest teats and more relaxed sphincter muscles that render ease of accessibility and establishment of infectious agent in the cows' udder **??**Radostitis et al., 2007). The association of parity with positivity for mastitis was found to be statistically significant (P<0.05). This finding was comparable with the previous reports (Tamirat, 2007;Mekibib et al., 2010;Haftu et al., 2012). This might be due to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without mastitis control program (Radostits et al., 2007) and cows having greater than 5calves were more affected than those with fewer and moderate calves **??**Zeryehun et al., 2013).

The relationship between the prevalence of mastitis on different lactation stage was studied, the result showed significantly higher infection (p<0.05) in cow with early (63.3%) and late lactation (52.7%) than cow with mid (30.5%) lactation stage. This result was agreed with G/mechael et al., (2013) and Biffa et al., (2005) who reported lactation stage had significant effect on the prevalence of mastitis in Ethiopia. Early stage and the late stage of lactation were the most susceptible stages. The mid lactation was lower. This could be due to the delayed diapedesis of neutrophils to mammary gland in recently calved cow and at late lactation there is decrement of neutrophil concentration when the cows reach to dry off (Workineh et al., 2002) and increased oxidative stress and reduced antioxidant defense mechanisms during early lactation (Sharmal et al., 2011). Moreover, absence of dry cow therapy regime could possibly be among the major factors contributing to higher prevalence at early lactation (Green et al., 2008), the high rates of new infection following drying off may be associated with the lack of flushing action of milking (Biffa et al., 2005).

The current study showed that the effect of milking hygienic practice was statistically significant difference (p<0.05) on the prevalence of bovine mastitis and infection rate was high in cows which not washed udder pre and post milking was (86.3%), followed by wash pre milking only 33.9% and lowest which wash pre and post milking 22.6%. The current study cross checked with previous findings (Lakew et al., 2009, Junaidu et al., 2011) both were reported that Cows at farms with poor milking hygiene standard are severely affected than those with good milking hygiene practices. The absence of udder washing, increased exposure and transmission of pathogens during milking (Kivaria et al., 2004), Whereas under Ethiopian conditions most of households use hand milking and washing hands, udder and teats before milking are not practiced, this could predispose dairy cows for pathogens (Bedane et al., 2012).

This current study showed that out of the 127 samples taken and growth 49/127 (38.5%) were found be positive for cultural isolation of streptococcus species. This result agreed with that of Bryson and Thomson 1990 at Bulawayo found to be 37% and 38% respectively and comparable with that of the report of Atyabi et al., 2006 Bishi (1998) who reported higher isolation rate (27%) for S. agalactiae. The reason for the higher isolation rate of this organism is the wide ecological distribution inside the mammary gland. In area where hand milking and improper use of drug is practiced to treat the mastitis cases, lack pre and post milking wash and teat dip, lack of dry cow's therapyand an adequate treatment clinical case. Its domination has been reported by many research scholars. S. agalactiae is adapted to survive in the udder an obligate agent of the mammary gland, S. agalactiae is a contagious cause of mastitis within a herd, sources of contagious mastitis are infected cows and transmission is from cow to cow, mainly at milking time through milking equipment, the milker's hands and contaminated wash cloths **??**Zoccone, 2006).

The present result indicated S. dysgalactiae isolated from milk sample (10.2) was similar with the previous findings of Ayano et al., 2013 who reported 10.6% atholota district. However, this finding was found to be higher when compared with Yohannis and Molla (2013), who reported 8.9% in and around walaitasodo, 7.2% by Duguma

et al., (2013), 5.6% by Kerro and Tareke (2003) and 0.5% by Bishi (1998), but lower than that of G/Michael et al., 2013 who reported 24% S.dysgactiae in and around ereka town. S. dysgalactiae are contagious pathogens were higher isolates in current study area might be due to lack of inter-cow hand washing and disinfection in the milking area andcontaminations of milkers' hands were spread of mastitis the present study agreed with previous study that spread of S. dysgalactiae between cows within dairy herds may occur directly or by way of the milking machine or environment (Younis et al., 2005).

Present study showed that Streptoccocus uberis (11.8%) was isolated which was in agreement with Ayano et al., 2013 who reported 12.1% at holeta district, but much higher than 4.23% by Kerro and Tareke (2003), 1.48% by Almaw et al., 2009 in and around bahirdar and (6.53%), by Mekebib et al. (2009) but lower than that of Zerihun (1996) and Iqbal et al. (2004) who reported in in Addis Ababa and Pakistan, 27% and 49.98%, respectively. Environmental streptococci may be due to poor housing facilities which predispose to the accumulation of feces on cows which could increase the rate of exposure of the teats and udder to the pathogens, not use dry cloth during milking, wash hand and material by common water, lack of dry therapy and improper of milking. This finding in line with many researches who reported s. uberis environmental factor during milking process, between milking, during the dry period and prior to parturition in first-lactation heifers and other environmental risk factor is housing and management practices such as contamination of bedding materials and exposure of teats to environmental streptococci **??**Hillarto et al., 2005).

18 V. Conclusion

The present study indicated overall prevalence of 49.2% which was a major health problem of dairy cows in the study area and undoubtedly would have an adverse effect on productivity of dairy industry. Relatively high prevalence of subclinical mastitis in dairy cattle of the study area due to lack of strategic control measures against the disease, lack of proper attention to health of the mammary glands, Lack of maintenance of strict hygiene and good sanitary environment contributory factors in the cause of clinical and subclinical mastitis. The major Streptoccocus species isolated was mainly Strepotoccocu agalactiae. Since the bacteria isolated from cows' milk samples was cause of both contagious and environmental mastitis the farmers should ensure strict personal hygiene and that of animals and sanitary condition of the farms should be improved and regular screening for the detection of subclinical mastitis should also be practiced. [1] [2] [3] [4]

1

Status	No. examined cow	CMT positive	Prevalence	X2	P-value
Sub clinical	384	160	41.66 %	3840.000	
Clinical	384	29	7.5%		
Total	384	189	49.2%		

a) Prevalence of Mastitis at Cows and Quarter Level

A total number of 384 lactating cows and 1536 quarter were included in this study. Out of which 189(49.2%) cows and 677(45.86%) quarter were be found positive for mastitis on CMT. Out of this 29 (7 160 (41.7%) were clinical and sub-clinical mastitis at cows level respectively and 6.8 % clinical and 38.86% sub-clinical mastitis at quarter levels(table 2).

Figure 1: Table 1 :

[1] © 2017 Global Journals Inc. (US)Year 2017

[2] Bovine Mastitis: Prevalence, Risk Factors and Isolation of Streptoccocus Species from Small Holders Dairy Farms in and Around Haramaya Town, Eastern Ethiopia

[3] © 2017 Global Journals Inc. (US)

[4] Bovine Mastitis: Prevalence, Risk Factors and Isolation of Streptoccocus Species from Small Holders Dairy Farms in and Around Haramaya Town, Eastern Ethiopia © 2017 Global Journals Inc. (US)

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Observation	No. Examined	No. Positive	Prevalence	Clinical mastitis. No.%	Sub-clinical mastitis. No. (%)		p-value
Cows	384	189	49.2	29 (7.5)	160 (41.7)	384	0.000
Quarter	1482	677	45.68	101(6.8)	576 (38.86)		

Figure 2: Table 2 :

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Quarter	No. examined teat	CMT positive quarter	Frequency %
Rear right	372	174	46.77%
Rear left	368	167	45.38%
Front left	372	170	45.69%
Front right	370	166	44.86%
Total	1482	677	45.68%

[Note: C]

Figure 3: Table 3 :

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Blind teat	No. examined teat	No. blind teat	No. Blind teat Clinical	No. blind teat Subclinical%	Proportion% of blind teat
Rear Right	1536	12	4	8	22.22
Rear Left	1536	16	4	12	29.62
Front Left	1536	12	3	9	22.22
Front Right	1536	14	4	10	25.92

Figure 4: Table 4 :

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Risk factors	Category.	No. examined	No. Positive	Prevalence %	X 2	P-value
Age	Young	202	86	42.5		
	adult	122	63	51.6	11.162	0.004
	Old	60	40	66.6		
Breed	Local	293	149	56.6	1.322	0.250
	Cross	91	40	43.9		
lactation stage	Early	115	74	64.3		
	Middle	108	33	30.5	27.464	0.000

Figure 5: Table 5 :

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	Late	161	85	52.7		
Parity	1-3(few)	198	85	42.9		
	4-6(moderat)	149	77	51.6	11.847	0.003
	?7(many	37	27	72.9		
Milking hygienic practice	Wash pre & post milking	141	32	22.6		
	Wash before milking	109	37	33.9		
	Not wash at all milking	134	120	86.3	137.079	0.000
Address	Haramaya town	108	49	45.3		
	IfaOromiya	101	50	49.5		
	Adele Waltaha	86	42	48.8	1.440	0.696
	TujiGebisa	89	48	53.9		
Total		384	189	49.2		

e) Bacterial Isolation and Identification

Species identified	Clinical	Subclinical	Proportion
Streptococcu, agalactiae	5	16	21(16.5%)
Streptoccocus, dysgalactiae	5	8	13(10.2%)
Streptococcus, uberis	2	13	15(11.8%)
Total	12	37	49(38.5%)

Figure 6: Table 6 :

Figure 7:

Figure 8:

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[Note: C]

Figure 9:

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