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A Study on Bovine Mastitis, Isolation and Identification of Staphylococcus Species in Dairy Farms of Dire Dawa City, Eastern Ethiopia

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Abstract: Staphylococcal mastitis is the commonest and economically the greatest concern wherever dairy farming is practiced. The chief reservoir of this bacterium is an infected udder. The organism is well adapted to survive in the udder and usually establishes mild sub clinical infection of long duration. A cross-sectional study was conducted from November 2014 to April 2015 to estimate prevalence of mastitis and to isolate and identify Staphylococcus species in lactating dairy cows in selected dairy farms of Dire Dawa city. A total of 334 milking cows (1336 quarters) tested using California Mastitis Test (CMT) and the prevalence at cow level was found to be 39.2% (131/334). Out of 131 positive cows, 84.73% (111/131) were subclinical mastitis cases and the rest 15.27% (20/131) were clinical mastitis. Out of 1336 quarters examined, 296 were found to be positive upon screening which account for the overall prevalence of 22.2% (296/1336) at quarter level. Out of 296 positive quarters, 18.91% (56/296) and 81.08% (240/296) cases were from clinical and subclinical forms respectively. From a total of 296 samples which were screening positive, 260 were found to be culture positive. From 260 culture positive samples, a total of 157 (60.4%) was positive for Staphylococcus species out of which the most prevalent being *Staphylococcus aureus (S. aureus)* (48.4%) followed by coagulase negative Staphylococcus (34.4%), *Staphylococcus intermedius* (12.7%) and *Staphylococcus hyicus* (4.5%). The presence of statistical significant association was assessed between the screening result and the risk factors like breed, parity and lactation stage and it was found to be statistically significant. Therefore, the result showed that prevalence significantly differed with breed ($P < 0.003$), parity ($P < 0.001$) and lactation stage ($P < 0.001$). Thus, prevalence was relatively higher in exotic (Holstein Friesian) than local and cross breed, in cows with many calves than those with moderate and few calves as well as in late and middle stage of lactation than early. In conclusion, this study revealed the importance of mastitis and associated bacterial pathogen in the study area.

Key words: Dairy Cows · Dire Dawa · Mastitis · Prevalence · Staphylococcus

INTRODUCTION

Mastitis (inflammation of the mammary gland) is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the world's dairy industry [1]. Staphylococcal mastitis is the commonest and economically the greatest concern wherever dairy farming is practiced. The chief reservoir of this bacterium is an infected udder. The organism is well adapted to survive in the udder and usually establishes mild sub clinical infection of long duration. Bacteria are shed into milk from infected quarters [2]. Transmission occurs mainly at milking time through contaminated milking machines, clothes and hands of milker's or machine operators [3].

Milk is a very nutritional food that is rich in carbohydrate, proteins, fats, vitamins and minerals. However, health risk to consumers can be associated with milk, due to the presence of zoonotic pathogens and antimicrobial drug residues [4]. The quality of milk may be

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lowered by a numbers of factors such as adulteration, contamination during and after milking and the presence of udder infections [5]. Bovine mastitis is a multifactorial disease and is one of the most difficult to control. Pathogenic organisms in milk can be derived from the cow itself, the human hand or the environment [4].

Clinical signs vary with the severity of the disease and generally include pain, heat and swelling of the affected quarter or half of the gland and abnormality of milk either as clots or flakes and wateriness of the liquid phase [6]. Bovine mastitis can be clinical with local (in some cases general) clinical signs and milk abnormalities or sub clinical with production losses and lowered milk quality. Efforts have only been concentrated on the treatment of clinical cases. As with most infectious disease, generally mastitis risk factors depend on three components; exposure to microbes, cow defense mechanism, environmental and management factors [7].

Owing to the heavy financial implications involved and the inevitable existence of latent infection, mastitis is obviously an important factor that limits dairy production. The disease should be studied as it causes financial loss as a result of reduced milk yield, discarded milk following antibiotic therapy, veterinary expense and culling mastitic cows [8]. Generally, bovine mastitis often follows a number of major factors involving the cow, the pathogen and the environment. The general predisposing factors influencing the occurrence of mastitis are hygiene, milking equipment and techniques, housing conditions, breed, level of production, the shape of the udder and teats, season and bacteria present in the environment [9].

Staphylococcus is a genus of Gram positive non-spore-forming cocci belonging to the family Micrococcaceae that are often found as normal human microbiota of the skin and nasal cavity. Staphylococcus is always facultative anaerobic. When stained, it will be seen in small clusters (staphylo = cluster). Staphylococcus is usually either beta hemolytic or not hemolytic at all (called gamma hemolysis). Pathogenic Staphylococci can produce a variety of virulence factors, including toxins, coagulase, leucocidins and hydrolytic enzymes that can damage host tissues. Staphylococcus species are a versatile pathogen of humans and animals that causes a wide variety of diseases. In human, it causes diseases that range from mild skin infection to more severe diseases such as pneumonia and septicemia [10].

In animals, it is commonly associated with mastitis leading to contamination of milk and dairy products. In the last few decades, Staphylococcal food poisoning has been reported as third cause of food-borne illnesses in the world. Among the foods implicated in Staphylococcal food poisoning, milk, dairy products and meats, particularly handled foods, play a vital role since entero-toxigenic strains of Staphylococcus species have been commonly isolated in them [10].

Staphylococcus species intra mammary infections *(*IMIs) are usually diagnosed by specific techniques in a microbiology laboratory. They are gram-positive cocci, which are catalase and coagulase positive. The numbers of Staphylococcus species found in the milk of an infected cow often shows cyclic variation. Bacterial numbers in milk may be high for a while, followed by an intervening period with much lower to non-detectable numbers of bacteria. A negative result or very low numbers of Staphylococcus species might be found in an infected cow when milk samples are collected during the period when the numbers of bacteria are at their lowest (or non-detectable) levels. A single negative result is not proof that a cow is uninfected with Staphylococcus species. A more accurate determination of a cow's infection status could be obtained by testing 2 or 3 milk samples collected on different days. Multidrug-resistant staphylococcal isolates such as Methicillin-Resistant *S. aureus* (MRSA) were isolated primarily from human samples, but such isolates were detected in animal samples [11, 12].

Thus, the transfer of Staphylococcus species between humans and cows may result in serious problems. Antimicrobial resistance is a main public health worry worldwide. Public hazards associated with the consumption of antibiotic contaminated milk could be allergic responses, changes in intestinal flora and development of antibiotic resistant pathogenic bacteria [13]. The expansion of resistance both in human and animal bacterial pathogens has been allied with the widespread remedial use of antimicrobials or with their administration as growth promoters in animals. Further transfer of antimicrobial resistant bacteria to humans via the food chain has been reported [14].

In this context, Staphylococcus present in milk may serve as a reservoir for human infections, thus allowing these microorganisms to persist and spread in the community. Up to now, many researchers have focused on the spread of resistant Staphylococcus species in clinical setting [10, 15].

Mastitis, as a disease, has received little attention in Ethiopia, especially the sub clinical form which is mainly caused by Staphylococcus species [16,17]. Even though

some investigations have been conducted in other areas of the country, there is lack of information on the major causes of bovine mastitis in Dire Dawa city especially on Staphylococcus species. Therefore, the objectives of the present research were to estimate prevalence of mastitis, to isolate and identify Staphylococcus species in lactating dairy cows in selected dairy farms of Dire Dawa city.

MATERIAL AND METHODS

Study Area: The current study was conducted in selected dairy farms of Dire Dawa city from November 2014 to April 2015. Dire Dawa is situated in the eastern part of Ethiopia at about 515km of Addis Ababa. The area is located between 9° 27' North and9°49' East latitudes and 41°38' North and 42° 19'East longitude. The rain fall pattern of the area is characterized by small rainy season from February to May and heavy rainy season from July to September. The dry season extends from October to January. The mean annual rain fall in the study area varies from 550mm in the lowland northern part to above 850mm in the southern mountain. The monthly mean minimum and maximum temperature ranges from 14.5°C to 34.6°C respectively. The entire territory of DDAC rests on an elevation ranging between 950m.a.s.l. in the north east to 2260m.a.s.l.in the south west. Using the1500m contour as a line of separation, two agro-ecological zones, the kola (below 1500m) and Woinadega (above 1500m) have been recognized [18].

Study Design: A cross-sectional study was conducted from November 2014 to April 2015 to estimate the prevalence of mastitis, isolate and identify staphylococcus species from selected dairy farms of Dire Dawa city, Eastern Ethiopia.

Study Animals: The study animals were all lactating dairy cows found in the randomly selected cow's population from conveniently selected dairy farms in Dire Dawa city, Eastern Ethiopia.

Ethical Consideration: Investigators treated animals with kindness and took proper care by minimizing discomfort, distress or pain. They assumed that all procedures which would cause pain in human beings may cause pain in study animals. The required procedures were conducted by qualified and experienced persons [19]. The ethical clearance was obtained from Wollega University ethical review board.

Sample Size Determination: For estimation of the prevalence of mastitis, since there was a work done in the study area, the sample size was determined by assuming the expected prevalence to be 32% with the 95% confidence level and desired precision of 5% using the formula described by Thrusfield [20].

$$n=\frac{(1.96^2[Pexpe(1-Pexpe)])}{d^2}=334$$

Where:
n= required sample size
Pexpe= expected prevalence
d= desired absolute precision
$Z^2=1.96^2$

Accordingly, the minimum sample size required was 334 lactating cows.

Study Methodology

Data Collection: The Data collected was recorded on the appropriate format designed for this purpose. Data regarding the different potential risk factors (age, breed, parity, lactation stage and previous history of mastitis) was collected for the cows from farm records when available and by interviewing the farm owner when not. Clinical examination of the udder, screening using the California mastitis test (CMT) and bacteriological examination were undertaken.

Clinical Inspection of the Udder: The udders of the study cows were examined visually and by palpation for presence of clinical mastitis. During examination attention was paid to cardinal signs of inflammation, symmetry, size and consistency of udder quarters [9].

California Mastitis Test (CMT): The California mastitis test (CMT) was conducted to diagnose the presence of subclinical mastitis and it was carried out according to the procedures given by Quinn *et al.*[21]. The udder of the cows to be tested was cleaned with warm water and antiseptics and dried with clean towel. Then the first few drops were discarded from each quarter. Following this squirt of milk from each quarter of udder, milk samples were poured in to four shallow cups in the CMT paddle and equal amount of CMT reagent was added to each cup and gentle circular motion was applied to the mixture on the horizontal plane. Based on the thickness of the gel

formed by CMT reagent and milk mixture, test results were scored as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive) (Annex 1). Milk samples with test result of CMT 1 to 3, were classified as evidence of subclinical mastitis [21, 9].

Detection of Mastitis: 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 [22].

Microbial Investigation of Mastitis

Milk Sample Collection: The milk sample was taken from cows not treated early with either intra mammary or systematic antimicrobial agents. For good collection of sample, the teat was wiped thoroughly with 75% ethyl alcohol. The sterile collection bottle was used and the first stream of milk from each quarter was discarded. The milk sample then held in an ice box for transportation to the laboratory. In laboratory samples were cultured immediately or stored at $+4^0$C [22].

Methods of Transportation and Storage of Samples: After collection of the milk sample, all samples were clearly labeled with the appropriate identification of the cows identification number, quarter using permanent marker on the test tube and all samples were transported with ice box to the Dire Dawa veterinary diagnostic laboratory and Haramaya University Veterinary laboratory accordingly without delay and it was processed immediately [7].

Preparation of Culture Media: To prepare media for bacterial culture, the manufacturer's instructions were followed. The common media that was used during the study include: blood agar, mannitol salt agar (Oxiod Hampshire, England), Edward medium (Oxiod Hampshire, England), Eosin methylene blue medium (Oxiod, Hampshire, England) and Trypton Soya broth (Oxiod Hampshire, England) media were used [7].

Culture: Before milking, milk samples were collected aseptically for microbiological culture, according to the procedures of the National Mastitis Council, 1999. Culturing of milk sample collected from individual cows, in search for mastitis producing organisms in standard of examination for mastitis [9]. All milk samples from clinical mastitis and subclinical mastitis which gave a positive reaction with California Test was submitted to laboratory and cultured on: Blood Agar, Nutrient Agar and Mannitol Salt Agar, all the Petri plate that contain this agar was incubated at 37 °C for about 24 - 48hrs [23].

Biochemical Tests: For the primary isolation and identification of mastitis causing microorganisms, colony size, shape, color, pigmentation, hemolytic characteristic and Gram's reaction were performed. After these colonies were sub cultured to different media, such as Mannitol salt agar, Trypton Soya Broth, Edward's medium (Oxiod Hampshire, England), Eosin methylene blue medium (EMB) (Oxiod, Hampshire, England), etc to get a pure culture. The biochemical test used for diagnosis of Staphylococcus species include: Catalase Test, Oxidase test, Coagulase Test, Hemolysis on blood agar, Gelatin Liquefaction Test (Gelatinase), Sugar Fermentation Test (Mannitol, Lactose, Mannose, Xylose, Trehalose, Sucrose, Maltose). The procedures for the identified pathogens were referred from Quinn *et al.* [7].

Data Management and Analysis: Descriptive statistics were used to summarize the generated data on the rate which was collected through, clinical inspection, CMT, isolation and identification Staphylococcus species. The obtained data was entered to Microsoft office excel 2007 program and analyzed using the statistical data analysis of STATA version 11.0. A statistically significant association between variables is considered to exist if the p value is < 0.05.

RESULTS

A total of 334 milking cows (1336 quarters) were tested using California Mastitis Test (CMT) and clinical examination of the udder. The overall prevalence of mastitis at cow level was found to be 39.2% (131/334) (Table 1).

From a total of 131 screening positive cows, the prevalence of clinical and subclinical mastitis at cow level were 15.27% (20/131) and 84.73% (111/131), respectively. Out of 1336 quarters examined, the overall quarter level prevalence was 22.2% (296/1336) from which clinical and subclinical mastitis at quarter level were 18.91% (56/296) and 81.09% (240/296), respectively (Table 2).

Table 1: Screening result and frequency of the study animals by farm, parity and lactation stage

			Result		
Characteristics	Frequency	Percent	Positive	Negative	Total
Farm					
1	27	8.08	17 (62.96%)	10 (37.04%)	27(100%)
2	28	8.38	23 (82.14%)	5 (17.86%)	28(100%)
3	25	7.49	21 (84.00%)	4 (16.00%)	25(100%)
4	50	14.97	15 (30.00%)	35(70.00%)	50(100%)
5	55	16.47	15 (27.27%)	40(72.73%)	55(100%)
6	70	20.96	23 (32.86%)	47 (67.14%)	70(100%)
7	30	8.98	6 (20.00%)	24 (80.00%)	30(100%)
8	25	7.49	6 (24.00%)	19 (76.00%)	25(100%)
9	24	7.19	5 (20.83%)	19 (79.17%)	24(100%)
Breed					
Cross	202	60.48	57(28.22%)	145 (71.78%)	202(100%)
Exotic	45	13.47	32 (71.11%)	13 (28.29%)	45(100%)
Local	87	26.05	42 (48.28%)	45 (51.72%)	87(100%)
Parity					
1-2	211	63.17	33 (15.64%)	178 (84.36%)	211(100%)
3-4	90	26.95	65 (72.22%)	25 (27.77%)	90(100%)
>4	33	9.88	33 (100.00%)	0 (0.00%)	33(100%)
Lactation					
Early	123	36.83	17(13.82%)	106 (86.18%)	123(100%)
Middle	107	32.04	36 (33.64%)	71(66.36%)	107(100%)
Late	104	31.14	78 (75.00%)	26 (25.00%)	104(100%)
Total	334	100%	131(39.22%)	203 (60.78%)	334(100%)

Table 2: Prevalence of clinical and subclinical mastitis at cow and quarter level

Forms of mastitis	No. of Cow examined	Positive	%	No. of quarter examined	Positive	%
Clinical	334	20	15.27	1336	56	18.91
Subclinical	334	111	84.73	1336	240	81.08
Total	334	131	39.2	1336	296	22.2%

Table 3: Association between animal risk factors and prevalence of mastitis

Animal factors	Total No. examined	No. positive	Prevalence (%)	X^2	P-value
Breed					
Cross	202	57	28.2	-	-
Holstein Friesian	45	32	71.1	29.5	0.003
Local	87	42	48.3		
Parity					
Few(1-2calves)	211	33	15.6	-	-
Moderate(3-4calves)	90	65	72.2	126.2	0.001
Many (>4calves)	33	33	100		
Lactation stage					
Early (<4months)	123	17	13.8	-	-
Middle(4-8months)	107	36	33.6	91.3	0.062
Late (>months)	104	78	75.0		0.001
Total	334	131	39.2		

Table 4: Culture result from CMT positive quarters in clinical and subclinical mastitis

Mastitis type	No. examined	CMT positive	%	Culture positive	%
Clinical	296	56	4.2	56	100
Subclinical	296	240	18.0	204	85.0
Total	296	296	22.2	260	87.8

Table 5: Isolation result from 260 culture positive samples

Characteristics	Frequency	Percent
Staph		
Negative for Staph	103	39.6%
Positive for Staph	157	60.4%
Total	260	100%
Staph. Species		
CNS	54	34.4%
S. aureus	76	48.4%
S. hyicus	7	4.5%
S. intermedius	20	12.7%
Total	157	100%

Animal risk factors such as breed, parity and stage of lactation have significant effect (P = 0.003, P = 0.001 and P = 0.001) on the prevalence of mastitis respectively, (Table 4). The result showed that the prevalence of mastitis was significantly higher 32 (71.1%) in exotic breed (Holstein Friesian), followed by aged local 42 (48.3%) and cross breeds 57 (28.2%). Parity had also showed an effect on the occurrence of mastitis. The prevalence was higher in animals with higher number of births followed by cows with parity of 3 to 4 and those with parity of 1 to 2. Lactation stage had association with the occurrence of mastitis in which the prevalence was higher 78 (75%) in late stage, followed by middle and early stages of lactation (Table 3).

Out of 296 (22.2%) CMT positive samples, 260 (87.8%) were culture positive as it is indicated in the table below (Table 4).

Microbiological investigation for isolation of Staphylococcus species from 260 culture positive samples showed that the prevalence of isolated species were grouped into *S. aureus* with 76 (48.4%) isolates, *S. intermedius* with 20 (12.7%) isolates, *S. hyicus* 7 (4.5%) and coagulase negative staphylococcus (CNS) with 54 (34.4%) isolates (Table 5).

DISCUSSION

The present study showed an overall prevalence of 39.2% comparable with that of Biffa, Etana and Fekadu [24] in and around Addis Ababa, Biru [25], in Adami Tullu district, Darsema [26], in Central part of Ethiopia and Getahun [27], in Alemaya who reported 38.9%, 35.7%, 39.5% and 36.9% respectively. This prevalence is relatively higher than the reports of Tolla [28] 24.09% in Northern Shoa (Sellale) area. This finding is lower than those of Zerihun [29] elsewhere in Ethiopia in cross bred dairy cattle who reported 68.10%. As mastitis is a complex disease involving interactions of various factors such as managemental and husbandry, environmental conditions, animal risk factors and causative agents, its prevalence will vary [9].

The prevalence of clinical mastitis in this study was 15.27% which is comparable to the reports done in different dairy farms: in dairy farms in Dire Dawa Administrative Council and Eastern Hararghe Zone, 15.1% by Biffa [30]; in and around Sebeta in Ethiopia (16.11%) by Hundera, Ademe and Sintayehu [16] ; in two major state owned dairy farms at Rapi and Debre Zeit, Ethiopia 21% by Workineh *et al.* [31]. This finding was higher than those reported: in Dire Dawa Autonomous and East Hararge Administration Regions 3.54% by Darsema [26]; in and around Gondar 4.4% by Tewedros [32]; in and around Mekelle 6.55% by Wudu [33]; in three states dairy farms around Addis Ababa 7% by Yirgalem [34] in Bahir Dar 3.9% by Gizat, Ademe and Yilkal [35] and in central high lands of Ethiopia 6.6% by Mungube [36]. While it was lower than reports done in selected areas of southern Ethiopia 37% by Sori, Zerihun and Abdicho [37]. In case of sub clinical mastitis the prevalence at cow level (33.2%) obtained in this study was comparable with the finding reported by Sori, Zerihun and Abdicho [37]. However, lower than those of Biffa [30] who reported 54.4%. Since, environmental factors play significant role, the prevalence of sub-clinical mastitis varies in dairy animals. In this study sub clinical mastitis has been found to be higher than clinical mastitis. This could be attributed to the little attention given to subclinical mastitis while treating clinical cases. A similar observation of the dominance of subclinical mastitis was observed by several studies [31, 38].

Quarter prevalence of mastitis (22.2%) found in this study was comparable with the finding of Biffa, Etana and Fekadu [24] who reported quarter prevalence rate of 28.20%, but lower than the report made by Nesru [39], who reported 44.80%.

From the 296 CMT positive quarter milk samples, 260 (87.8%) was culture positive, while 36 (12.2%) were bacteriologically negative, which is in line with the results of Aregaw [40], who reported 18% bacteriologcally negative samples. The failure of isolation of bacterial agents from CMT positive quarter milk is due the predominance of subclinical mastitis in the area. Lack of stringency in provision of therapy can possibly change the clinical cases into subclinical mastitis. The administration of antibiotics can suppress the bacterial agents and inhibit their growth in the media. It could also be due to spontaneous elimination of infection, intermittent shedding of pathogens and intracellular location of pathogen and the presence of inhibitory substances in milk [9].

Animal risk factors such as breed, parity and stage of lactation have significant effect (P = 0.003, P = 0.001 and P = 0.001) on the prevalence of mastitis respectively (Table 4). The result showed that the prevalence of mastitis was significantly higher 32 (71.1%) in exotic breed (Holstein Friesian), followed by aged local 42 (48.3%) and cross breeds 57 (28.2%). Parity also had effect on the occurrence of mastitis. The prevalence was higher in animals with higher number of births followed by cows with parity of 3 to 4 and those with parity of 1 to 2 in that order. Lactation stage had association with the occurrence of mastitis in which the prevalence was higher 78 (75%) in late stage, followed by middle and early stages of lactation, respectively.

Subclinical mastitis was high in all breeds compared to clinical mastitis. The prevalence of subclinical mastitis at cow level in the present study (33.2%) was comparable to the reports done in different dairy farms: in Bahir Dar 34.4% by Gizat, Ademe and Yilkal [35] and Bishi [41] who reported 34.3%; Abaineh and Sintayehu [42], 34.6%. But the present finding is by far lower than the reports of Kerro and Tareke [43] in local, Friesian and Jersey cows in Ethiopia (63%): Machang and Muyungi [44] in Tanzania (67%) and Kivaria, Noordhuizen and Kapaga [45], in lactating cows in smallholder farms in Tanzania.

The significant effect of stage of lactation on prevalence of subclinical mastitis in this study was 13.8%, 33.6% and 75.0% in early, mid and late lactation, respectively. Out of 296 (22.2%) CMT positive samples, 260 (87.8%) were culture positive. The result obtained from bacteriological analysis for isolation of Staphylococcus species from 260 culture positive samples showed that the predominant species was *S. aureus*. The prevalence of isolated species in the present study revealed 76 (48.4%) *S. aureus* isolates, 20 (12.7%) *S. intermedius* isolates, 7 (4.5%) *S. hyicus* isolates and 54 (34.4%) coagulase negative staphylococcus (CNS) isolates. The high prevalence of *S. aureus* in this study is comparable with that of Milne *et al.*[46] who reported 44.4% in Sebeta, but higher than 9% reported by Kivaria, Noordhuizen and Kapaga [35]. Similar study conducted in Jamaica and India by Zingeser *et al.* [47] and Barbuddhe, Chakcrkan and Sundaran [48] indicated lower *S. aurues* isolates which accounted 27% and 23.2%, respectively

The result of coagulase negative staphylococcus (CNS) in present study accounted 34.4%, which is lower than 42% [49] finding. However, it is higher than 10% prevalence reported by Milne *et al* .[46]. CNS is regarded as minor pathogen and normally considered as normal inhabitants of bovine udder [50].

CONCLUSION

The present study revealed that mastitis was a major health problem of dairy cows in the study area and undoubtedly would have an adverse effect on productivity of dairy industry. Hence warrants serious attention. The major bacterial from mastitic milk samples was Staphylococcus species mainly *Staphylococcus aureus* and coagulase negative Staphylococcus. Since the current study was limited with time and resource it needs further investigation.

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REFERENCES

1. Wallenberg, G., H. Vanderpoel and J. Vanior, 2002. Viral infection and bovine Mastitis. J. Vet. Micro., 88: 27-45.
2. Tsegaye, A., 1988. Study on bovine mastitis in and around Holeta. Addis Ababa University, Faculty of Veterinary Medicine, DebereZeit, Ethiopia, (Unpublished DVM thesis).
3. Radostitis, O., D. Blood and C. Gay, 1994. Veterinary Medicine: A text book of the diseases of cattle, sheep, pigs, goats and horses. 8thed. Bailliere Tindall: London, pp: 563-613.
4. Bradely, A., 2002. Bovine mastitis an evolving disease. J. Vet., 164: 116-128.
5. Esron, D., E. Karimuebo, T. Lughano, R. Kusiluka, M. Melegela and M. Kapaa, 2005. Study on Mastitis, milk quality and health risk associated with consumption of milk from pastoral herds in Dodoma and Morgora region, Tanzania, J. Vet. Sci. 6: 213-221.
6. Mifflin, M., 2004. Bovine Mastitis- Definition of bovine mastitis in Medical Dictionary, the free dictionary by Farlex, pp: 15-20.
7. Quinn, P., J. Market, M. Carter, W. Donnelly and F. Leonard, 2002. Veterinary Microbiology and Microbial disease. Blackwell science, London, pp: 465-472.

8. Hillerton, J., 1987. Summer Mastitis: Vector transmission or not? Parasitology, Today, 3: 121-122.
9. Radostits, O., C. Gay, W. Hinch cliff and P. constable, 2007. Veterinary medicine a text book of the disease of cattle Horses, sheep pigs and goats. 10th ed. London: Sounders, pp: 1462-1464.
10. Ateba, C., M. Mbewe, M. Moneoang and C. Bezuidenhout, 2010. Antibiotic-resistant Staphylococcus aureus isolated from milk in the Mafikeng Area, North West province, South Africa. S Afr. J. Sci., 106: 12-13.
11. Asao, T., Y. Kumeda, T. Kawai, T. Shibata, H. Oda, K. Haruki, H. Nakazawa and S. Kozaki, 2003. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: Estimation of enterotoxin A in the incriminated milk and powdered skim milk. Epidemiol. Infect., 130: 33-40.
12. Lee, J., J. Jeong, Y. Park, S. Choi, Y. Kim, J. Chae, J. Moon, H. Park, S. Kim and K. Eos, 2004. Evaluation of the Methicillin-Resistant Staphylococcus aureus (MRSA): Screen latex agglutination test for detection of MRSA of animal origin. J. Clin. Microbiol., 42: 2780-2782.
13. Thirapatsakun, T., 1999. Mastitis management in: small holder dairying in the tropics. In: Hunt and Chantalakhana (Eds). ILRI, Kenya, Nairobi, pp: 299-339.
14. Angulo, F., V. Nargund and T. Chiller, 2004. Evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans and human health consequences of such resistance. J. Vet. Med., 51: 374-379.
15. De silva ciombra, M., M. Silva-carvalho, H. Wisplinghoff, G. Hall, S. Tallent, S. Wallace, M. Edmond, A. Figueredo and R. Wenzel, 2003. Clonal spread of Methicillin- Resistant Staphylococcus aureus in a large geographic area of the United States. J. Hosp. Infect., 53: 103-110.
16. Hundera, S., Z. Ademe and A. Sintayehu, 2005. Dairy cattle mastitis in and around Sebeta, Ethiopia. Intern. J. Appl. Vet. Med., 3: 1525-1530.
17. Mekonnen, H., S. Workineh, M. Bayleyegne, A. Moges and K. Tadele, 2005. Antimicrobial susceptibility profile of mastitis isolates from cows in three major Ethiopian dairies. Med. Vet., 176: 391-394.
18. DDAEPA, 2011. Dire Dawa administration program of adaptation to climate change. DDAEPA, Dire Dawa, Ethiopia. pp: 5-6.
19. Sahni, S.K., 2000. Indian national science academy: Guidelines for care and use of animals in scientific research. New delhi: Bahadur Shah Zafar Marg; 110 002 and printed at bengal offset works, 335, khajoor road, karol bagh, newdelhi 110005.
20. Thrusfield, M., 2005. Veterinary epidemiology, 3rd edition. Blackwell science. Ltd. Oxford. pp: 232-234.
21. Quinn, P., M. Carter, B. Markey and G. Carter, 1999. Clinical Veterinary Microbiology, Elsevier Ltd, London, pp: 118-344.
22. NMC, 1990. Microbiological procedures for the diagnosis of Bovine udder infection. 3rd ed. Arlington, VA: National mastitis council, Inc.
23. Koneman, E., S. Allen, W. Janda and P. Schreckenberger, 2007. Color Atlas and Text Book Diagnostic Microbiology .5th Lippincott Willams and Wilkins .Washington DC. Winn, Jr. U.S.A.
24. Biffa, D., D. Etana and B. Fekadu, 2005 Factors Associated with udder infections in lactating cows in Southern Ethiopia Inter. J. Appl. Res. Vet. Med., 3: 189-198.
25. Biru, G., 1989. Major Bacterial causing Bovine mastitis and their sensitively to common Antibiotics. Eth J. Agri. Sc., 11: 47-54.
26. Darsema, G., 1991. A survey of Bovine mastitis in different dairy farms: Dire Dawa Autonomous and East Harerge Administrative Regions. DVM Thesis: Faculty of Veterinary Medicine, Addis.
27. Getahun, K., 2006. Bovine mastitis and Antibiotic resistance patterns of major pathogens in small holder dairy forms in central high land of Ethiopia. MSC thesis: Debre-Zeit: Factory of Veterinary medicine, Addis Ababa university, Ethiopia.
28. Tolla, T., 1996. Bovine Mastitis in Indigenous Zebu and Borena-Holstein Crosses in Southern Wollo. Thesis, Debre Zeit: Faculty of Veterinary Medicine, Addis Ababa University: Ethiopia, pp: 25-27.
29. Zerihun, T., 1996. A study on bovine sub clinical mastitis at Stela dairy farm. Thesis, Debrezeit: Faculty of Veterinary Medicine, Addis Ababa University: Ethiopia. pp: 25-27.
30. Biffa, D., 1994. A study on the prevalence of bovine mastitis in indigenous zebu cattle and Jersey breed in Wollaita Sodo, Characterization and in vitro drug sensitivity of isolates. DVM Thesis, Addis Ababa Univ. FVM, Debrezeit, Ethiopia.

31. Workineh, S., M. Bayleyegne, H. Mekonnen and L. Potgieter, 2002. Prevalence and aetiology of mastitis in cow from two major Ethiopian dairies. Trop. Anim. Hlth Prod., 34: 19-25.
32. Tewedros, 2007. Prevalence and economic significance of bovine mastitis in and around Gondar. DVM thesis Mekelle University Faculty of veterinary medicine, Ethiopia.
33. Wudu, T., 1999. Study on bovine mastitis in and around Mekelle. DVM thesis, Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia.
34. Yirgalem, G., 1987. A survey on the prevalence and etiology of bovine mastitis in three state dairy farms around Addis Ababa. DVM thesis, Addis Ababa University, Faculty of' Veterinary medicine. Ethiopia.
35. Gizat, A., AZ. deme and A. Yilkal, 2007. Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. Trop. Anim. Health Prod., 40(6): 427-32.
36. Mungube, E., 2001. Management and economics of dairy cow mastitis in Urban and peri- Urban areas of Addis Ababa University, Debre Zeit, Ethiopia, Msc thesis.
37. Sori, T., A. Zerihun and S. Abdicho, 2005. Dairy Cattle Mastitis in and around Sebeta, Ethiopia. Int. J. Appl. Res. Vet. Med., 3(4): 332-338.
38. Sori, T., J. Hussien and M. Bitew, 2011. Prevalence and susceptibility assay of Staphylococcus aureus isolated from bovine mastitis in Dairy Farms of Jimma Town, South West Ethiopia. J. Anim. Vet. Adv., 10(6): 745-749.
39. Nesru, H., 1999. A cross-sectional and longitudinal study of bovine mastitis in urban and peri-urban Dairy System in the Addis Ababa region, M. Sc thesis, Free University of Berlin and Addis Ababa University, Ethiopia.
40. Aregaw, M., 1992. Incidence of mastitis and its influence on milk yield and composition in Debrezeit. DVM Thesis, Addis Ababa Univ. FVM, Debrezeit, Ethiopia.
41. Bishi, A.B., 1998. Cross-sectional study and longitudinal prospective study of bovine clinical and subclinical mastitis in peri-urban and urban dairy production system in Addis Ababa Region, Ethiopia. Msc Thesis, Addis Ababa Univ. Free Univ. Berlin, Germany.
42. Abaineh, D. and A. Sintayehu, 2001. Treatment trial of subclinical mastitis with the herd presicaria seneglense. Trop. Anim. Health. Prod., 33: 511-519.
43. Kerro, O. and F. Tareke, 2003. Bovine Mastitis in Selected Areas of Southern Ethiopia. Trop. Anim. Hlth. Prod., 35: 197-205.
44. Machang, U. and L. Muyungi, 1998. The occurrence of streptococci mastitis in dairy farm in Morogoro area, Tanzania. Bul. Anim. health and Prod. Afr., 36: 190-193.
45. Kivaria, F.M., J.P. Noordhuizen and A.M. Kapaga, 2004. Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania, J.Trop. Anim. Health. Prod., 1: 581-592.
46. Milne, M.H., D.C. Barrett, J.L. Fitzpatrick and A.M. Biggs, 2002. Prevalence and a etiology of clinical mastitis on dairy farms in Devon. Vet. Rec., 158: 241-243.
47. Zingeser, J., Y. Day, V. Lopez, G. Grant, I. Bryan, M. Kearney and Me Hugh-Jones, 1991. National Survey of Clinical and Subclinical Mastitis in Jamaican Dairy Herds. Trop. Anim. Health Prod., 23: 2-10.
48. Barbuddhe, S.B., E.B. Chakcrkan and R.N.S. Sundaran, 2001. Studies on incidence and etiology of bovine mastitis in Goa region. Indian J. Comparison Microbiol. Immunol. Infect. Dis., 22: 164-165.
49. Hussein, N., 1999. Cross sectional and longitudinal study of bovine mastitis in urban and peri urban dairy systems in the Addis Ababa region, Ethiopia, MSc Thesis, Faculty of Veterinary Medicine, Addis Ababa University School of Graduate Studies and Freie Universitat, Berlin, Berlin.
50. Gentilini, E., G. Denamiel, A. Betancor, A. Rebuelto and M. Rodriguez, 2002. Antimicrobial susceptibility of Coagulase Negative Staphylococcus isolated from bovine mastitis. J. Dairy Sci., 85(8): 1913-1917.