



Prevalence of Bovine Mastitis and Assessment of Risk Factors in and Around Wolayta Sodo, Ethiopia

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Abstract: A cross sectional study was conducted in and around Woliata Sodd in different dairy breeds (jersey, indigenous zebu, and high grade Holstein breed) from November 2013 to May 2014 to estimate the prevalence of mastitis and to assess the associated risk factors at Sodd regional veterinary laboratory. A total of 386 lactating cows of which 216 indigenous zebu, 88 jersey, 82 high grade Holsteins underwent a clinical examination and Californian Mastitis Test (CMT) was performed. Moreover, those positive for the clinical and sub clinical mastitis were subjected to bacteriological tests. N. 134 (34.7%) were found to be positive for either clinical (38.80%) or subclinical mastitis (61.19%). Of the 966 quarters examined, 21.94% were found positive to mastitis. The prevalence of mastitis differed significantly among the breeds ($P < 0.000$) with 54.8% in high grade Holstein cows, 44.3% in jersey cows and 23.1% in indigenous zebus. However, there was no significant difference among breeds as to quarters. With respect to lactation stage, the prevalence of mastitis was 43.85% at early lactation (1-120 days), 32.80% at mid lactation (121-240 days), and 23.8% at end lactation (above 240 days). Moreover, there was a significant difference between lactation ($P < 0.000$). The prevalence of mastitis in various group was found to be increasing with parity number ($r = 0.8$) with highest prevalence at lactation stage number six and above. Of the total 134 CMT positive and clinically positive udder quarter sample analyzed microbiologically, 111 (82.8%) were culturally positive and 23 (17.2%) were negative. In positive samples, *Staphylococcus* accounts for 43.54%, *Streptococcus* 28.89%, *Coliform* 19.35% respectively, of the total 124 bacteria isolated. Based on the result obtained, recommendations were forwarded to build up awareness among dairy cow owners and further investigation with special emphasis on economic losses.

Keywords: Woliata Sodd, Bovine Mastitis, Prevalence, CMT, Bacteriological Examination, Risk Factors

1. Introduction

Mastitis is the inflammation of mammary glands that result from injurious agents including pathogenic microorganism, trauma, and chemical irritants. In dairy cows it is always caused by microorganisms, usually bacteria, but other organism including yeast, mycoplasma and even algae occasionally cause mastitis. The great majority of infections are caused by species belonging to the genera *Staphylococci*, *Streptococci*, and several gram negative bacteria. The common pathogens comprise contagious bacteria, mainly coliforms and some species of streptococci that are commonly present in the environment and may reach the teat end from that source [1].

Mastitis is most often sub clinical in nature, but can manifest itself in either mild or peracute clinical forms. The causation and severity of the disease involves a complex relationship of the host, agent and the environments [1, 2]. Mastitis is a major cause of economic importance decrease production and of productivity. Heavy losses can occur due to mastitis milk, treatment costs, discarding of milk with antibiotics, lower price for quality of milk, and death from severe inflammations [1, 3].

Milk from a mastitic animal can create potential danger for food poisoning, the interference with manufacturing processes, and for the spread of zoonotic disease such as tuberculosis, brucellosis, streptococcal sore throat. In addition, antibiotic residue infects, and milk of mastitis treated animals is a matter of public health concern [1].

In Ethiopia, the limited information available [8] indicates that bacteria mastitis is one of the major facing all dairy farms in the Country. Moreover, Mohammed [9] in his study on the bacterial causes of bovine mastitis in Zebu, Holstein dairy cows of Wondogenet all of southern Ethiopia found a quarter infection rate of 16% by potentially pathogenic bacteria however, economic losses due to mastitis in Ethiopia are not known. According to [10] in their assessment of 10 state dairy cows more than 10% of cows in most farms in Ethiopia have at least one blind quarter. In addition, [11] in their assessment of 10 state dairy farms near Addis Ababa point out that mastitis accounts for major economic losses. In general, regarding the occurrence and distribution of major causative agents of the disease, in Ethiopia in general, and in southern regions in particular, is inadequate, moreover, not enough investigations have been carried out to ascertain the effects of different risk factors on the prevalence of mastitis. Therefore, by considering all the above information this study set out the following objectives: to estimate the prevalence of bovine mastitis in Woliata Soddo; to isolate and identify major potentially pathogenic bacteria that cause bovine mastitis and to recommend the appropriate control and prevention methods that are applicable in the study areas.

2. Materials and Methods

2.1. Study Area

This study was carried out in Woliata Soddo town, in regional veterinary laboratory from November 2013 to May, 2014. Woliata Soddo town is located in the SNNPR about 383 km from Addis Ababa. The area is bounded with Damot gale woreda to the north, Humbo Woreda to the south, Damotwoide woreda to east and Damotsore woreda to the west. Its altitude ranges from 1650 to 2980 (m.a.s.l), it receives an annual rain fall of 100 – 1200 mm and an annual temperature of 25 – 35°C. The area is woinadega (mid altitude) with altitude below 1600 and livestock population found in the Woliata Soddot own comprises about 128,919 cattle, 29191 sheep, 4606 equines and 55278 poultry in Woliata Soddo zone [12].

2.2. Study Population and Their Management System

Here, we studied indigenous zebus from around Sodo, jersey cows from Sodo dairy farm, and high grades Holstein Frisian from the dairy farm in the sodo area and in the Sodo town. Indigenous zebu animals in peasant farm are reared mainly for meat, milk production, and the generation of cash income and the provision of draft power. They are not supplemented except during dry season when farmers offer their animals straw of various crops and pasture grasses in a cut, and carry form. They usually graze on communal pastures where transmission of contagious disease is facilitated. The dairy farm at Soddo had relatively improved management system; jersey cows were housed in a free stall in which each animal was tied by chain. Pregnant cow were transferred to a maternity house a few days before

parturition, generally without any preparation. Calves aging up to 6 months were penned separately. Cows were allowed to graze on native or improved pastures or both growth on the faced grazing land of the farm and watered with hay or concentrates mainly during the dry season. Generally, problem associated with inadequate nutrition were uncommon, milking was conducted twice a day

2.3. Study Methodology

2.3.1. Study Design

A cross sectional study design was conducted from November 2011 to May 2012.

2.3.2. Sample Size Determination and Sampling Technique

A total of 386 cows, of which 216 indigenous zebu, 88 jerseys and 82 high grade Holstein Friesian were sampled using the formula on [13]. The cattle were grouped into lactating cow (in milk), breed as indigenous zebu, jersey and high grade Holstein Friesian, lactation stage into early lactation (1-120 days) mid lactation (121-240 days) late lactation (above 240 days) and parity number as 1st, 2nd, 3rd, 4th, 5th, 6th and above 6. Milking sample was collected hygienically after each quarter was washed with clean water and soap and then cleaned with a swap dipped in 70% alcohol while extruding the external sphincter by pressure to ensure that dirt and wax were removed from the orifice. Ten ml of milk were collected in a test tube and held in oblique manner to prevent contamination of samples by falling particles.

2.4. Data Collection

During the sampling of animals for the present study, the breed, parity number, status of mammary gland, stage of lactation or lactation period were noted, and so were clinical findings (e.g., palpable and visible abnormalities of the udder, nature and appearance of milk secretion).

Questionnaire: A preliminary survey was conducted in the specified study areas and adjoining localities during initial period of the study. The questionnaire targeted mainly dairy farms and livestock owners; it was aimed at generating basic information on livestock management system, nutrition, hygienic practices, disease detection, housing, prevention and control measures.

Diagnosis of mastitis: Although mastitis in an individual cow may be obvious, it is more often a sub clinical case or disease and clinical. Therefore tests for the detection of changes in the udder or the milk caused by mastitis are necessary. In this study criteria for the detection of mastitis included: 1) Physical examination of udder quarter for signs of inflammation by a thorough clinical inspection and palpation; and 2) Detection of high level of leukocytes in milk using the Californian mastitis test (CMT).

Microbiological procedure: All positive samples were analyzed microbiologically by CMT and clinical examination according to [14]. Samples from individual quarter were centrifuged at 3000 revolution for 15 minutes and supernatant was discarded. Standard loop full of 0.01ml of milk was then removed from the sediment and cultured on

blood agar plates containing 7% of sheep blood, Maconkey agar plates or Edward's agar plates. Inoculation of plates performed with a 0.01 ml wire loop. The original milk samples were also incubated at the same temperature and cultured similarly 24 hours after bacterial growth was identified and recorded, at 24 and 48 hours of incubation.

At the end of this period, identification of isolated bacterial was made on the basis of morphology, color and size of the colonies, presence or absence of hemolysis and gram stain. The identification of bacterial isolates up to the species level was made by sub culturing a single pure colony and conducting the following tests:

Staphylococcus species and *micrococci* were identified and differentiated by gram staining, colony morphology, catalase test, tube coagulase test using human plasma, ability to produce hemolysis, ability to ferment maltose, and manitol.

Streptococcus species were identified and differentiated by colony morphology catalase test, growth characteristic on Edward's media, ability to produce hemolysis CAMP test.

Coliform species were identified and differentiated by colony morphology on the Maconkey agar; gram staining, motility test, indole test, triple sugar iron agar for the detection of lactose and glucose fermentation and hydrogen gas production.

2.5. Statistical Analysis

The data collected was filled into microsoft excel and then analyzed with Chi-square and P- value using SPSS version 20.

3. Result

3.1. Questionnaire

In the farms included in this study no strip cup was used and dry therapy and post milk disinfection were not practiced. Clinically detected cases of mastitis have been treated by parental injection of locally available drug, most of the time by penicillin, streptomycin, and oxytetracyclines. Animal at the Soddo dairy farms and in farmers hand had high tick infestation, despite regular treatment with steladone. As regards nutrition, almost all dairy cows, especially those in peasant's farms, were kept on dry roughage except during rainy season when green feeds are available. Type of housing ranged from open kraals to free stalls in which cow were tied with chains.

3.2. Clinical Examination

Of the total 386 cows examined, 134 (34.7%) were found to be positive for mastitis: 52 (38.8%) clinical and 82 (61.1%) sub clinical mastitis. Out of 966 quarters, 212 (21.94%) were found to be affected: 83 (39.15%) clinically and 139 (65.56%) sub clinically affected. In the clinical cases, 12 blood tinged milk with sign of inflammation in the affected quarter, 20 had watery without sign of inflammation in the quarters, 13 milk contained pus with swollen quarter mostly. Seven animals had acute mastitis with signs of inflammation in the quarter and severe inflammation. Among all animal examined, 105 blind quarters were found and those that had an indurate mass due to fibrosis were common in most cows.

3.3. Prevalence of Mastitis

Clinical examination and screening test results indicate an overall prevalence rate of 34.7%. The prevalence rate differed significantly among breeds ($p < 0.05$).

Table 1. Prevalence of mastitis by breed in and around Wolaita Soddo.

Breed	No of animal examined	No. of affected	No. of not affected	prevalence
Indigenous zebu	216	50	166	23.1%
Jersey	88	39	49	44.3%
High-grade Holstein	82	45	37	54.8%
Total	386	134	252	34.7%

$\chi^2 = 31.04$; $P = 0.000$

Quarter prevalence of mastitis is found to be 25% in high grade Holstien and 20.82% in indigenous zebu with no significant difference among quarters of the three breeds.

Table 2. Quarter prevalence of mastitis by breed.

Breed	No. of quarter examined	Noof affected	No of not affected	prevalence
Indigenous zebu	485	101	384	20.82%
Jersey	281	61	220	21.70%
High grade holstien	200	50	150	25%
Total	966	212	754	21.94%

$\chi^2 = 1.43$; $P = 0.49$

The highest prevalence is seen in early lactation (1-120 days) in which out of 228 animal examined 100 (43.85%) were found positive and the lowest prevalence is seen in end lactation (<240 days) 23%.

Table 3. Prevalence of mastitis in different lactation stage.

Stage of lactation	No. of examined	No. of affected	No. of not affected	Total
Beginning (1-120 days)	228	100 (43.85%)	128 (56.14%)	228
Middle (120-240days)	137	44 (32.25%)	93 (68.19%)	137
End (<240 days)	21	5 (23.80%)	16 (76.19%)	21
Total	386	149	237	386

$\chi^2= 7.03; P= 0.000$

The highest prevalence (64.51%) is observed at a parity number 6 and above and the lowest prevalence (26.08%) observed at first parity number which is the prevalence raised with an increase in parity number ($r= 0.8$).

Table 4. Prevalence of mastitis in various parity groups.

Parity number	No. of cowexamined	no. of mastitic animal	None mastitic animal	Prevalence %
1	115	30	85	26.08%
2	93	17	76	39.54%
3	75	29	46	38.66%
4	54	28	26	51.85%
5	18	10	8	55.55%
6 and above	31	20	11	64.51%

The prevalence of 41.66% was seen in animals with lesions and/or tick infestation on teat and /or udder skin as compared to animals without lesions and/or tick infestation, in which the prevalence was 25.88%.

Table 5. Prevalence of mastitis in relation to predisposing factors.

Cow	Infected animal	None-infected	Total
With lesion and /or tick on skin or udder	90 (41.66%)	126 (58.33)	216
Without lesion and tick infestation on skin /teat or udder	44 (25.88%)	126 (74.11)	170
Total	134	252	386

$\chi^2= 12.52; P= 0.02$

In the present study, the highest prevalence was seen in housed animals as compared to pastured cows.

Table 6. Prevalence of mastitis by management system.

Management	Animals examined	No. of affected	None affected	Prevalence
Pastured	216	50	166	23.14%
Housed	170	84	86	49.41%
Total	386	134	252	34.7%

$\chi^2= 35.42; P= 0.000$

3.4. Milk Bacteriology Results

Bacteriological examination was carried out on all CMT and clinically positive udder quarter samples to identify the causative organism involved in the disease. The organisms were identified based on colony morphology, gram stain, hemolysis and

biochemical test for species identification. The differentiation of microorganisms was carried out according to [14].

Out of 134 positive animals, 52 were clinically infected while 82 were sub-clinically infected. From 134 positive animals only 111 is culturally positive.

Table 7. Prevalence of clinical and sub clinical mastitis.

Infection status	Clinical mastitis	Sub clinical	Total
Infected	52 (38.15%)	82 (61.1%)	134
None-infected	126 (32.64)	126 (32.64%)	252
Total	178	208	386

Of all culturally positive animals, 46 were from clinical and 65 from subclinical cases.

Table 8. Staphylococcus identification.

hemolysis	Catalase	Tube cougaluse	Manitol test	0-F test	Frequency of isolation	Species of bacteria isolated
+ve	+ve	+ve	+ve	F	54	<i>S. aureous</i>
-ve	+ve	+ve	-ve	F	2	<i>S. hyicus</i>
-ve	+ve	-ve	-ve	F	3	<i>S. epidermides</i>

+ve=positive;-ve=negative; F= fermentation; CMT= Californian mastitis test O-F= oxidation fermentation test; S=staphylococcus

Table 9. *Streptococcus* species identification and differentiation results.

Hemolysis	Catalase	CAMP test	Hydrolysis of aesculin on Edward media	frequency of isolation	Species of streptococcus identified
Alpha	-ve	+ve	-ve	10	<i>Strep. agalactiae</i>
Beta	-ve	+ve	-ve	6	<i>Strep. agalactiae</i>
Gamma	-ve	-ve	+ve	7	<i>Strep. dysagalactiae</i>
Beta	-ve	-ve	+ve	4	<i>Strep. dysagalactiae</i>

-ve= negative +ve=positive strep= streptococcus

Table 10. *Genera of bacteria isolated from clinical and sub clinical mastitis.*

Cases	Staphylococcus	Streptococcus	Coliform
Clinical	15	7	7
Sub-clinical	25	12	8
Total	40	19	15

Out of all clinical cases, 15 were caused by staphylococcus, 7 were caused by *Streptococcus*, and 7 by coliform species. Out of all subclinical cases, 25 were by *Staphylococcus*, 12 by *Streptococcus* and 8 by coliform species.

4. Discussion

The study was carried out to determine the prevalence of bovine mastitis in Woliata Soddo town, in the southern part of Ethiopia. Overall, the disease showed a prevalence of 34.7% in cows and 21.94% in quarters. This finding agrees with the general assertion by [1] who indicates that in most countries the incidence rate is 40% in cows and 25% in quarters, irrespective of the cause. The infection rate in cows also closely agrees with the findings of [4] who reported a prevalence of 45.8% in Sudan and [15] who observed prevalence 45.5% in Ethiopia. It was lower than findings of [4] who reported a quarter infection rate of 38.8% in Sudan. The variation in overall prevalence may be due to the effects of environment, agent, and host factors involved in the causation of mastitis. In this study, unhygienic milking procedure and poor management may be predisposing factors for increased infection rate. Particularly, management varies from place to place and this might contribute to the observed difference.

A significant difference in the prevalence rate was detected among zebu, jersey and high grade Holstein cows ($P < 0.05$). This observed by different authors, [16] found significantly higher incidence rate than the Holstein Frisian and Hariana crosses were more susceptible to sub-clinical mastitis than Jersey-holstien crosses. Further [18] found a significantly higher prevalence rate in zebu holstien crosses is in zebu breed in Kombolcha in Ethiopia. This variation may due to the influence of the magnitude of the milk yield on the occurrence of mastitis. A similar observation is made by [1] who asserts that high yielding cows are more susceptible. This may be due to the ease with which injuries are more susceptible are sustained in large udders in which the foci for the entrance of pathogens are created and the high milk yield stress that may up set the defense system of the animals. The local zebu in which the prevalence rate was significantly less than the two breed (jersey and Holstein) were under potential risk of mastitis in that milking practice did not involve

hygienic procedure, lesion and /or tick infestation on udder and /or teat skin was found to be greater as compared to other breeds.

In this study prevalence of 61.19% in sub-clinical mastitis and 38.80% in clinical form. The magnitude of clinical cases in agreement with the annual incidence of 41.2% in dairy herds of England. The difference between sub-clinical and clinical mastitis has been reported by [19] in Sudan [5], in Ghana [20], 46%, 20% and 41.67% respectively. The effect of lactation stage on the prevalence of mastitis was assessed and it was found that a prevalence rate of 43.85% during early lactation (1-120 days) significantly different ($P < 0.05$) higher than 32.25% of mid-lactation (121-240 days) and 23.25% of end lactation. This finding agrees with that of [21] who reported high incidence in sahiwal cows during early lactation in India.

According to [22] different types of physiological stress (early lactation) may impair immunity, resulting in increase in the number of new infections of mammary gland. [23] suggest that lactation usually is associated with acute mastitis mainly due to reduced resistance resulting from delayed diapedesis of neutrophils in the mammary glands. This observation may account for the high prevalence rate seen in early lactation in this study. Effects lesion and /or tick infestation of udder and /or teat skin on the prevalence of mastitis was assessed and it was found that animals without this lesion and/or tick infestation on udder and /or teat skin had a prevalence of rate of 41.66% as compared to animals without this factors 25.88%. This finding was consistent with a report made by [18], [20], [24] and, [3], the possible for the high incidence on this group of animals was teat lesions that were frequently colonized by staphylococci and lesser extent by streptococcus organism.

The effects of the management system on the prevalence of mastitis assessed 49.41% in housed and 23.14% in pastured cows. This findings agrees with finding of [25] who found a significantly higher prevalence rate in housed animals, this has substantiated by an easy transmission of organism from infected to healthy ones. The result obtained from microbiological analysis of the sample revealed that the predominant organism isolated from the predominant organism isolated from the result were staphylococcus aureus which accounted for 43.54% out of the total 124 isolated.

This finding was consistent with those of [6], [26], all of whom found *Staphylococcus aureus* as predominant isolates from bovine mastitis.

The predominance of *Staphylococcus aureus* could attribute to the wide ecological distribution of the organism on intra mammary and skin of the udder and frequent colonization of the eroded and injured skin on the teat and/or udder of the cows. [1] maintain that this organism is well adapted to survive in the udder and usually establish mild infection of long duration from it shed thorough milk facilitating transmission to healthy animals mainly during unhygienic milking procedures. The isolation rate might be due to poor hygienic conditions, absence of dry therapy, and lack of milking teat dipping and low culling rate of animals with repeated attacks of mastitis. In the genus *Staphylococcus*, *S. epidermidis*, and *S. hyicus* accounted for isolation rate of 2.41% and 1.61% respectively. In this study, coagulase negative *Staphylococcus* was taken as *S. epidermidis* but other species *S. hyicus*.

Escherichia coli was isolated recovery rate of 16.12%, this results agrees [27] who reported isolation rate of 11.5% it is relatively higher than Demelash who reported isolation rate of 3.14%, and *Klebsiella aerogenes* occur with isolation rate of 3.23%. This coliform organism are ubiquitous in the environment of dairy cows with manure being the major source [3]. These pathogens are commonly described as possess a greater ability than other common mastitis pathogens to survive and multiply in extra mammary sites and is no uniform method of control proven effective under experimental condition.

Streptococcus agalactiae isolated 12%, agree with those of [28], [29], and [27] who found an isolation rates of 14.3%, 10.3% and 13.5%, respectively. The result was lower than that of [30] found to be 37% and 38% respectively. The relatively high isolation of this organism in this study may due to poor milking time hygiene, absence of post milking teat dipping, lack of proper treatment for clinically infected animals, lack of use of dry period therapy and absence culling of non-responding of dry period therapy cows. *Streptococcus dysagalactiae* and *Streptococcus uberis* accounts for isolation rates of 8.87% and 4% respectively.

Most of the infections were sub-clinical and the majority did not receive treatment as results, most of the infections act as source of infections for other cow in the herd. Farmers and herd managers only concerned with clinical mastitis and often unaware of prevalence of the infection in the resulting economic loss. Control of mastitis in dairy herd can be accomplished in parts with aid of antibiotics however, antibiotic treatment is not entire answer to mastitic problem moreover, indiscriminate use of drug especially penicillin, and streptomycin which may have potential effect on the development of resistant strain bacteria. Therefore, in the light of these remarks the following recommendations are forwarded: Strict hygienic procedure should followed in dairy farms with regard to milking practices; Culling of chronically infected cows with or without blind quarters should be made so that potential source of pathogens could

be eliminated; Farmer's awareness of the disease must be promoted through educating farmers by implementing short term training, extension workers, and models farmers and further investigation should be continued with special emphasis on economic losses predisposing factors to convince the farmers about economic losses precipitated by the disease on dairy production.

Author Contribution

JSh: conception of the research idea, designing data collection, interpretation of the results and drafting the manuscript. IT: data collection and drafting the manuscript. The authors read and approved the final manuscript.

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Competing Interests

All authors have declared that no competing interests exist.

References

- [1] Radostitis, O. M.; Leslie, K. E and Fetrow, J: Mastitis control in dairy herds: Herd health food animal production medicine 2nd ed W. B Saunders company Philadelphia; 1994: 229-273.
- [2] Schalm, FL, and Radiostitis, O. M: The occurrence of streptococci mastitis in dairy farm in Mogogora, Tanzania Bulletin of animal health and production in Africa; 1994: 36: 2 196-129.
- [3] Schalm, O. carrol, E. S and Jain, N. C: Bovine mastitis. Lea and Fiebiger, Philadelphia: USA. 1971: 1.302.
- [4] Abdelrahim, A. I., Shommerin, A. I., Suliman, H. B and shad. lad SAI: Prevalence of mastitis in imported friesioan cows in Sudan: *Review production and veterinary medicine for tropical countries*; 1989: 42 (4) 512-514.
- [5] Tizard, I: Immunity in the mammary glandveterinary immunology, an introduction 4th ed. Saunderscompony, Philadelphia; 1992: 244-245.
- [6] Mohammed, I. E Mohammed, G. E and EL. DWNI OAO: A study on the incidence and etiology of bovine mastitis in Sudan, proceeding of the second scientific congress. Egyptian society of cattle disease, 5-7 December; 1993: 2: 326-336.
- [7] Machang, U. R. S and Muyungi, L: The occurrence of streptococcal mastitis in dairy farms in Mogogora area, Tanzania. Bulletin of animal health and production in Africa; 1988: 36: 2, 190-129.
- [8] Tsegaye. H. M: Study on bovine mastitis Addis Ababa University, Haremaya (Ethiopia) miscellaneous publication college of agriculture; 1972: 41-44.

- [9] Mohammed, A: Bacterial cause of bovine Mastitis in Wondogenet, Ethiopia jour of vet Med; 1996: 379-384.
- [10] Goshu, M, Kasahun, D. and Tesfaye, A: A study of state farm; Jour. 11 (1): 1985
- [11] Mekonnen. Franser, Clarencey: Mastitis. The merky veterinary manual 7th ed. Merck and co, incrahway, N.J.U.S.A; 1999: 686-692.
- [12] CSA, Basic data on agricultural resources, development potentials and constraints. Ethiopia: WZA Department of Agricultural Development (WZADAD); 2001: 4-44.
- [13] Thrusfield H: Veterinary epidemiology: Black well science 3rd ed; 1995: 237-240.
- [14] Cifrina, E., Alber, J. Gand Warren, W. M: Role of milk fractions, serum and divalent cations protection of mammary epithelial cell of cows against damage by staphylococcus aureus toxins. American journal of veterinary research; 1996: 3, 308.
- [15] Mangistu, A: Bacterial cause of bovine mastitis in Wondogenet, Ethiopia. Jour. Vet. Med; 1996: 379-384.
- [16] Smith, Cmary and Sherman, M David: Mammary gland and milk production: Goat medicine. Lea and Febiger Philadelphia; 1994: 465-487.
- [17] Schukken, Y. H., Grommers, F. J., Geer, D-van-de., Erb, H. N., Brand, a and van-de, Geer: Risk factors for clinical mastitis in herds with a low bulk somatic cell count: Journal of dairy science. Netherlands; 1990: 3463-3471.
- [18] Sol. J: Summer mastitis: epidemiology, etiology, prevention and therapy: Mastitis treatment. Making it work. proceeding of the third British mastitis conferen Ciba-giegy agro chemicals; UK; 1990: 28-35.
- [19] Bagadis, H. O: The etiology of Bovine mastitis in three different areas in the Sudan Khartoum University, Sudan; 1978: 18, 86.
- [20] Demalash, B. A: A study on the incidence of bovine mastitis in indigenous zebu cattle and jersey in Woliatasoddo. DVM thesis Faculty of Addis Ababa university; 1994.
- [21] Prem, chand; Behar, G. D.; chakaravarty. A. K and chand, p: Comparative incidence of mastitis in relation to certain factors in cattle, and buffaloes: Indian journal of animal science; 1995: 65; 1, 12-14.
- [22] Hamman: A study of the incidence of bovine mastitis in different cooperative dairy farmin chilalo awraja, Arsi region DVM thesis Faculty of veterinary medicine Addis ababa university; 1987.
- [23] Smith, K. L.; Todhunter, D. A and Shoenberger, P. S: Sympostium; Environmental effects on cow health and performance environmental mastitis cause, prevalence and prevention: Journal of dairy science; 1985: 68: 5-8-1531-1553.
- [24] Tarekegn: Bovine mastitis in indigenous Arsi breed and Arsi Holstien crosses: prevalence: DVM thesis Faculty of veterinary medicine Addis Ababa University; 1996.
- [25] Frandos, R. D, and spurgeon, T. L: Anatomy of mammary glands: anatomy and physiology of 5th edition lea and Febiger, Philadelphia; 1992: 466-473.
- [26] Fred, C. N: Chloranphinicol treatment of staphylococcal mastitis: Low southern western veterinarian; 1960: 13: 114-118.
- [27] Tiruneh, Z: Study on bovine sub clinical mastitis in Asella dairy farm prevalence, DVM thesis, Faculty of vet. Medicine. Addis Ababa University, 1996.
- [28] Odongo, M. O and Ambani AIA: Microorganism isolated from isolated from bovine milk sample submitted veterinary diagnostic laboratory kebetu Kenya; 1989.
- [29] Gezahegn, S: Prevalence and etiology of sub clinical mastitis in Freisian –indigenous zebu crosses in bahirdar. DVM thesis. Faculty of veterinary medicine, Addis Ababa university; 1996.
- [30] Bryson, R. M, and Thomson, J. W: Laboratory and field control of clinical mastitis in dairy cows around Bulawayo: Journal of South African association; 1990: 3 203.