ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

BACTERIOLOGICAL LOAD IN MILK AND PREVALENCE OF SELECTED DISEASES OF INTENSIFICATION IN ADDIS ABABA DAIRY FARMS

BY

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A Thesis Submitted to the Faculty of Veterinary Medicine, Addis Ababa University in Partial Fulfillment of the Requirement of the Degree of Master of Science in Tropical Animal health and Production

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ARAB	Addis Ababa region agriculture beauro
CI	confidence interval
CIDT	Comparative intradermal Tuberculin Test
CMT	Califomic mastitis test
CSA	Central Statistical authority
ES	Ethiopian standards
FAO	Food and agricultural organization of the united nations
GMP	Good Management Practice
HACCP	Hazard Analysis Critical Control Point
IDF	International dairy federation
ISO	International standards organization
LMA	livestock marketing authority
ML	mili liter
MM	mili meter
MRT	Milk ring test
OIE	Office international epizootics (international office of epizootics)
PPD	Purified protein derivative
P-value	probability value of rejecting a null hypothesis while it must be accepted
SPC	Standard plate count

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ABSTRACT

A cross sectional investigation of the bacteriological quality of milk and the prevalence of selected diseases of intensification was carried out on 31 intensive farms which were selected as clusters. Data was collected using questionnaire survey, farm observation, clinical examination, CMT, bacterial count, comparative intradermal tuberculin test and milk ring test. The results of this study showed that none of the modern farms (n=31) kept records except one farm. All except three farms were poorly drained and less hygienic. Univariate ANOVA indicated that there was no significant variation observed in the mean log cfu/ml of SPC between the different categories of hygienic practices, availability of potable water and herd size ($p>0.05$). Herd level prevalence of clinical and subclinical mastitis was 38.75% and 100%, respectively. The prevalence of subclinical mastitis at quarter, cow and herd level was 83.03% (n=1042), 81.8% (n=327) and 100% (n=31), respectively. The prevalence of clinical mastitis at quarter, cow and herd was 3.4% (n=52), 6.5% (n=26) and 38.7% (n=12), respectively. Moreover, 7% of cows were with at least one blind teat and the quarter level teat blindness is 2.81%. Quarter level clinical mastitis was significantly associated with parity number ($p<0.05$), udder hygiene ($p<0.05$), farm hygiene ($p<0.05$), stage of lactation ($p<0.05$) and herd size ($p<0.001$) while only parity ($p<0.05$), stage of lactation ($p<0.01$) and herd size ($p<0.001$) were significantly associated to sub-clinical mastitis. The highest prevalence of quarter level clinical mastitis was observed in early (1.6%, n = 16) and late parities (1.5%, n =24), in cows with poor udder cleanliness (2.13%, n =34), in farms with poor hygiene (2.0%, n =32), at late lactation stage (1.8%) and in farms with herd size ranging from 26 to 40 (1.56%, n= 6). On the other hand, the prevalence of subclinical mastitis significantly increased with parity number and stages of lactation and the highest quarter level prevalence was observed in the herd size ranging from 26-40. On the other hand, the overall prevalence of bovine tuberculosis was 62.66% and the disease occurred in significantly higher rates in herds with larger number of animals, in farms with poor ventilation and in cows with 3 and 4 parities. But, age, sex and stage of lactation had no significant association with the prevalence of the disease in the present study. The results of multivariate logistic regression showed that infection of cows by bovine tuberculosis was more of the function of herd size and the ventilation status. The prevalence of bovine brucellosis was 12.9% (n=4) and there was significant association between the herd level prevalence of bovine brucellosis and the abortion

history in the herds. On account of these results it is concluded that bacterial quality of raw milk produced and marketed by intensive dairy farms in Addis Ababa is of inferior quality and possible risk to public health from zoonotic diseases. Therefore, it is recommended that national milk quality standards and regulations demarcating the minimum operational environments of the routine husbandry practices and health requirements of dairy animals should be instituted.

Key words: Bacterial load; Bovine brucellosis; Dairy farms; Intensive system; Bovine mastitis; Milk quality; Bovine tuberculosis

1. INTRODUCTION

Next to blood, milk is the most complex bioactive substance which is the normal secretion of the mammary glands and is one of the nutritionally balanced food items gifted to mammals (McMannama and Neville, 2003). Milk from an ideally healthy cow never contain any microorganism and is virtually sterile as it is synthesized and secreted by the lacteal cells of the cow. Thus, it is so honored by mammalian evolutionary development to promote the growth, development and the health of the new-borne (Black *et al.,* 2002; Johansson, 2002; Vokk *et al.,* 2005). However, unless dairy products are of standard grades and qualities in terms of nutritional composition, microbiological profile and chemical safety, they can impose big risk to the public health due to milk borne zoonotic infections, intoxications and drug residues (Noordhuizen, 2003a; Noordhuizen, 2003b).

Form the very nature of its composition, milk can act as a very good substrate to the growth and replication of bacteria, in fact owing to the fluid nature of it and balanced nutrient composition. It is one of the most vulnerable items to be accessed by microorganisms from various sources like the production environment and the cows themselves (Paul, *et al.,* 1992); be it from endogenous source such as infected cow (Zerihun,1996; Godefay and Molla, 2000; Alehegn, 2004) or exogenous source such as the production environment (Srairi, *et al.,* 2006). Bacteria such as *Mycobacterium, Salmonellae, Brucellae, Staphylococcus and Escherichia coli* are major milk-borne pathogens frequently incriminated to endanger human health and take considerable research priority in many parts of the world including Ethiopia (Robinson, 1985; Yilkal, 1998; Godefay and Molla, 2000; Alehegn, 2004; Ameni *et al.,* 2007).

In view of the above problems, the primary objective of dairy production in most countries is centered on two major pillars of the sector namely, a balanced mix of quality and quantity. In countries with poor milk production and marketing practices and with traditional husbandry, veterinary and sanitary concepts, it is expected that very high initial viable bacterial counts can be found in raw milk posing a health risk to the general public and shorter shelf life of the product (Hagstad and Hubbert, 1986; Noordhuizen, 2003b).

The main determinants of milk quality are the health status of the cows, the hygienic status of the farming premise and the sanitary and phytosanitary practices in the milk production channel, storage and transportation process (Donald, 1998). Thus for efficient and quality dairy production, investigation of the herd health for the prevalence status of diseases of economic and public health importance that can be transmitted through milk and its products is required (Nordhuizen *et al.,* 2005).

With little opportunity to get pasteurized milk and a wide spread habit of consumption and commercialization of raw milk, people in developing countries are at higher risk of infection from milk-borne diseases mainly bovine tuberculosis and brucellosis (Moda, *et al.,* 1996; Kangethe *et al.,* 2000; Weinhaipul *et al.,* 2000; Mendez *et al.,* 2006). Poor quality milk is also an important economic concern to the dairy business and industry as poor quality product will fetch discouraging values and would also be wasted due to rapid microbial spoilage and be unfit to certain technical requirements of milk processing (Henri *et al.,* 2003). In addition to this, it is important to consider the knowledge attitude and practice in dairy farms in the light of the overall food safety concerns. This is required because the pre-harvest safety and quality controls must be based on "good management practices" (GMP) while post-harvest quality control activities must apply both GMP and Hazard Analysis and Critical Control Point (HACCP) principles in which the bench mark information could be tapped from surveys involving the triads of herd health, management of the husbandry and the feeding practices (Noordhuizen, 2003a; Noordhuizen, 2003b).

Recent studies conducted in Morocco show that milk hygienic quality is highly variable from farm to farm (Srairi *et al*., 2006) which have been attributed to the difference in milking and rearing conditions in the farms. Similar results indicating poor bacteriological quality of milk as a function of poor husbandry, milking practices and higher prevalence and incidence of mastitis has been recorded by Chaye *et al*. (2004) and Alehegn (2004).

Of the dairy health problems known to date, infection of the milk gland by microbial pathogens is the number one factor limiting both the quality as well as the quantity of milk produced in dairy farms (Fual, 1983; Radostits, *et al.,* 1994). Many investigators have come up with positive

relationships of the bacterial load of the bulk of milk with the prevalence of mastitis in dairy herds (Auldist *et al.,* 1995; Auldist and Hubble, 1998). On the other hand, the occurrence of mastitis is highly influenced by a multitude of factors related to the animals, the environment, the etiologic agents and the management of the husbandry operation (Radostits *et al.,* 1994; Coulona *et al.,* 2002; David *et al.,* 2005; Dankor *et al.,* 2007). In consequence to these intercalated facts, the production of milk that conforms to the food safety regulations and legal standards is a very difficult task. Yet, it is an obligatory concern of all; producers, processing plants and consumers (Scoones and Wolmer, 2006).

Since dairy farms in the resource-limited countries like Ethiopia strive in the widespread presence of diseases and in compromised sanitary conditions, they produce milk of poor quality and higher public health risks (Cosivi *et al.,* 1998; Ameni *et al.,* 2000; Alehegn, 2004; Ameni *et al.,* 2007a). This is why many countries and concerned international organizations have set out minimum standards for the quality of milk at various stages of production, processing and distribution (Griffith, 1957; IDF, 1991; PMD, 1995).

Because of its perishable and delicate properties and health risks so far discussed, milk and its products should be produced and marketed under strict quality monitory and control procedures. But it is hardly possible to see any one of such concerns to monitor milk quality in Ethiopia. Therefore, information on the overall quality aspects of milk with reflections on the health status of the dairy herds, the bacteriological quality of bulk milk and the associated risk factors is required.

The present study was carried out, thus, with the following objectives:

- To study the microbiological quality of raw milk produced by intensive dairy farms in Addis Ababa;
- To determine the prevalence of bovine mastitis, tuberculosis and brucellosis in intensive dairy farms in Addis Ababa;
- To determine the risk factors associated with milk quality and prevalence of bovine mastitis, tuberculosis and brucellosis.

2. LITERARURE REVIEW

2.1. Urban and peri-urban dairy production

In Ethiopia, four major systems of milk production are distinguished and these are: pastoralism, highland smallholder, urban and pre-urban and intensive dairy farming systems (Mohamed *et al.*, 2003). The urban and peri-urban system is developed in and around major cities and towns, which have a high demand for milk (Azage and Alemu, 1998). Both the urban and peri-urban dairy systems are located near or in proximity of Addis Ababa and regional towns and take the advantage of the urban markets with a primary objective of selling milk as a means of additional cash income (ILCA, 1995; Azage *et al.,* 2000; Stephen *et al.,* 2006).

It is estimated that about 5,167 small, medium and large scale dairy farms producing about 35 million liters of milk annually are found in the Addis Ababa milk shed (Azage and Alemu, 1998). The size of the overall cattle population in the small, medium and large-scale farms in Addis Ababa is presented in Table 1. Yields are significantly higher in Addis Ababa due to the high incidence of crossbred and exotic cattle. Hybrid and exotic cows represent only 1.8% of total milking cows in Ethiopia but 47% in Addis Ababa (Stephen *et al.*, 2006).

The majority of the small-scale farmers found in three administrative sub-cities namely; Akaki-Kality, Nifas Silk-Lafto and Bole sub-cities have been organized into dairy cooperatives (ARAB, 2008). These farms supply their milk to either of the pasteurization plants in Addis Ababa or Sebeta. But part of milk produced by these cooperatives and that produced from the large scale dairy producers is supplied mainly to consumers in raw through the local/informal market channels (Brahu and Debra, 1991; Ketema and Tsehay, 1995).

There are no official rules and regulations to control the quality of milk produced and distributed to consumers in the city except at the Dairy Development Enterprise (DDE) which makes quality control on milk entering the processing plant for pasteurization, which is a very small proportion of the total amount of milk produced in the country (13%) (Ketema and Tsehay, 1995). The same authors have reported that of the total milk produced in the urban dairy system, 73 % is for

market, 10% is left for household consumption, 9.4 % goes to calves and 7.6 % is processed into butter and cottage cheese. Of the milk producers in this milk shed, the highest proportion of milk (71%) is distributed to consumers informally as raw milk without passing through any type of quality and safety tests (Yilkal, 1998).

Table 1. Population of different types of cattle in Addis Ababa City in 1996

Herd Composition	Exotic/Crosses	Indigenous	Total
Cows	14,045	9,177	23,222
Heifers	4,720	3,840	8,560
Female calves	4,404	3,815	8,219
Male calves	2,316	3,044	5,360
Bulls	588	671	1,259
Total cattle including oxen	27,249	31,319	58,566

Source: Getachew and Gashaw, 2001

2.2. Measures of milk quality

2.2.1. Bacteriological Quality

Milk is virtually sterile when it is synthesized in the healthy cow's udder (Linzell and Peaker, 1971) but due to its complex biochemical composition and high water activity, it serves as an excellent medium for the growth and multiplication of many kinds of microorganisms (James, 1996). Thus, it can be contaminated while it is within the udder, if the cow gets infected by bacterial agents as in the case of milk from mastitic cows (Faul, 1983; Quinn *et al.*, 1994; Fernandes *et al.*, 2004). It is usually contaminated by organisms free living in the environment or that are found on surfaces that come in contact to it during the process of milking (Fernandes *et al.*, 2004 and Courtenay *et al.*, 2005), processing (Elmagli and Ibtisam, 2006), storing and transporting (Abdel Moneim *et al.*, 2006).

Milk may contain both pathogenic and nonpathogenic organisms. Pathogenic organisms, which may come directly from the cow's udder, are species of *Staphylococcus*, *Streptococcus*, *Mycobacterium, Brucella, Escherchia, Corynebacterium,* and others which are known to cause disease to animals and man. Generally, bacteria in milk are classified in to three groups based on the temperature for optimal replication namely: psychrophilic, messophilic and thermophilic bacteria (Dogan and Boor, 2003). Psychrophilic organisms can grow at refrigeration temperatures and responsible to the spoilage of milk under cold storage. The psychrotrophic bacteria are killed by pasteurization, however, the enzymes they produce can survive and their importance in the dairy sectors is due to the economic loss from their spoiling actions on milk stored at lower temperatures there by reducing its shelf life. Thermophilic organisms are less harmful to health, but can cause changes in the organoleptic qualities of milk. They can endure heat and survive pasteurization. Mesophilic bacteria are those, which grow best at temperatures ranging from about 20 to 40 ^{o}c. These groups of bacteria include the pathogenic genera and are also capable of deteriorating the quality of milk (Hagstad and Hubbert, 1986). This group of bacteria is responsible for milk borne infections of human beings.

Owing to the wide spread occurrence of bacterial agents in milk, this item of food is subject to various standards and quality tests that can indicate how safe and nutritionally valuable a given dairy product is. These quality indicators include the standard plate count (SPC), total coliform count (TCC), Escherichia *coli count,* (EC), psychrophilic incubation (PIC), laboratory pasteurized count (LPC), and somatic cell count (SCC). In addition to these general bacteriological tests, milk is often subjected to more detailed laboratory analysis to isolate and identify specific pathogens to man and the cow itself (Donald, 1996; Rice and Bodman, 1997; Jay, 2000; Loir *et al.,* 2003; Kavaria *et al.,* 2004; Kessel *et al.,* 2004; Rosemini *et al.,* 2004). But the routinely applied method for investigation of bulk milk quality is the standard plate count and the somatic cell count (IDF, 1991).

Standard plate count (SPC)

The standard plate count (SPC), which is also called the aerobic plate count (APC) of raw milk gives an indication of the total number of aerobic bacteria present in the milk at the time of culturing the samples. Analysis of total bacterial count (TBC) may be performed using the bactocount equipment that applies the flow cytometry technique (Holm *et al.,* 2004). In the SPC method, milk samples are plated on standard plate count agar media and then incubated for 48 hrs at 32 ^{o}C to encourage bacterial growth. Single bacteria or clusters grow to become visible colonies that are then counted. All plate counts are expressed as the number of colony forming units (cfu) per milliliter of milk (Murphy, 1996).

A negative aspect of the standard plate count is the possible underestimation of the total bacterial quantity because a colony does not always originate from a single bacterium (Qiunn *et al.,* 1999) and it does not indicate the types of the bacterial species involved in the milk contamination. It is sensitive but also labor intensive and is inaccurate for high-count milk samples (Slaughuis, 1996). It is required that the samples be processed immediately after collection or within 24 hours of collection, if appropriate cooling facility is in place. However, the SPC is generally accepted as the most accurate and informative method of testing bacteriological quality of milk. Table 2 gives the grading system of raw milk based on its SPC.

Table 2. Grade of raw milk based on standard plate count

Bacterial count/ml	Grade
Not exceeding 200,000	Very good
200,000 – 1,000,000	Good
1,000,000-5,000,000	Fair
>5,000,000	Poor

Source: Kurwijila *et al*., 1992.

The Bactocount method is another method of enumerating the total bacterial number in raw milk based on the addition of ethidium bromide in to the sample to color bacterial nucleic acids in which dyed milk is injected into a capillary tube connected to an optic system that constantly receives a laser beam. When dyed milk passes through the system, each bacterium emits fluorescence that is captured by the optic system and determines the total number of bacteria in individual bacterial count (ibc) (Suhren and Walte, 1999; Barrientos *et al*. 2000; Barbano and Lymch, 2006). This type of analytic technique makes possible the utilization of bacteriostatic preservatives that decrease bacterial metabolic activity, extending useful life of milk samples used for TBC analysis.

Coli forms count

Coliform is not a biological classification, but a working definition given to a group of bacteria, which inhabit the intestinal tracts of human and animals (Jay, 2000). E coli are the most numerous coliforms in humans and animal intestines and are derived almost exclusively from these sources being excreted in large number with human excreta and animal droppings (Quinn *et al.,* 2002). They may be found in the soil, on vegetables and in untreated water (Dogan and Boor, 2003; Kessel *et al.,* 2004). It does not survive long in water and, therefore, it is the best indicator of recent human and animal faecal pollution. Its presence in milk indicates a potentially dangerous pollution; high counts a heavy or recent pollution and low counts a slight or more remote one (Jay, 2000).

Somatic cell counts (SCC)

Raw milk contains three types of cells; epithelial cells, macrophages and polymorphonuclear cells. The fist two can be found in uninfected udder while polymorphonulear cells and macrophages are found in high numbers only in infected udder (Radostits *et al.,* 1994; Quinn *et al.,* 2002). Due to this, the somatic cell count (SCC) is internationally recognized as a parameter for assessing both milk quality and udder heath (Degraaf *et al*., 1997). The so-called premium milk or grade "A" milk should have a SCC of less than 4.0×10^5cells/ml of milk.

Dye Reduction Test on Raw Milk

The principle of these tests is based on the effect of the bacteria on some reagents added to milk samples serving as an indirect indication of the bacteriological quality. These include: methylene blue reduction test, resazurine reduction test, alcohol test and clot on boiling test (Teka, 1997). In this test, a known dilution of the methylene blue solution is added to the milk sample and observation is made at fixed intervals until the blue color of the solution disappears (Teka, 1997). Normally, if the number of bacterial organisms is greater, the time required to decolorize the blue color is shorter.

Another test, whose procedure as well as the principle is similar to that for the methylene blue reduction test, is resazurin test. The only exception is that this test is quicker and the result is obtained in much less time (Teka, 1997). The resazurin dye imparts blue color to milk which when reduced to resorufin changes to pink and finally to white when reduced to dihydroresorufin. The time required for complete decolorization, reduction of the resazurin and the degree of colour change is directly related to the number of bacterial organisms in the milk (Ombui *et al*., 1995; Teka, 1997). A disc reading value of 4 and above for 10 minutes resazurin test indicates good quality but while reading value of less than 4 at 10 minutes indicates poor quality milk (Ombui *et al*., 1995).

Alcohol test can be performed on milk to check its freshness particularly at milk collection centers and other field conditions (Kirwijila *et al*., 1992; ISO, 2000). When milk contains more acids, calcium or magnesium compounds in greater than normal concentration, it coagulates on the addition of alcohol. This fact is the basis of alcohol test, which furnishes a means of judging the quality of milk. Various other tests can be done on milk as summarized in Table 3.

Table 3. List of other tests used to test the quality of milk

Type of the Test	Remark
Titrable acidity test	If the milk sourness is 4 to 5 Soxhilet-Henkel Degree SH^0, it indicates that either the milk is adulterated or there is mastitis
Phosphatase test	Phosphatase, an enzyme, normally present in raw milk, is inactivated by heat. A positive test will indicate that the milk is not properly pasteurized.
Sedimentation test	Leaving milk standing still for 15-20 minutes and observing the formation of any sediment
Ph determination	Measures the amount of acid(H+ ion produced by bacteria multiplying in milk
Catalase test	This measures the activity of the enzyme catalase content of which depends up on the number of cells in milk. Hence the increased activity of this enzyme indicates mastitis.
Specific gravity	Normal unadulterated cows milk ranges between 1.026 and 1.032 at 20 ^{0}C measured by lactometer.
Freezing test	The normal freezing point of milk is between -0.50 and -0.61^{0}C
Conductivity	Milk from mastitic cows will have increased conductivity due to the increase in the ionic contents of the milk
Organoleptic tests	general appearance, cleanliness, colour and smell of the fresh milk should be checked

Source: Hagstad and Hubert (1986)

2.2.2. Biochemical quality of milk

Constitutional variation of milk is not only due to species difference but also it is the function of feed regimens (Heristov *et al.,* 1999) and genotype within the same species of animals (Bonfoh *et al.,* 2000; Afif *et al.,* 2007). In addition to the food nutrients, milk also contains immunoglobulins that are transferred directly from the blood serum into the milk and enzymes produced from the mammary tissue which impart unique biological properties to the milk. Some of the bioactive molecules in milk and their function include the following:

Lactoferrin:

This is an iron-binding glycoprotein that is found in the milk, saliva, and other body fluids of mammals. Research by Still *et al.* (1990), Edde *et al.* (2001) and Teraguchi *et al.* (1995) has shown that purified lactoferrin have broad spectrum antibacterial activity against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and other food borne pathogens and spoilage organisms by sequestering environmental iron which serves as a growth factor for bacteria.

Lactoperoxidase:

It is one of most heat stable enzymes found in milk. It has bacteriostatic effect when it is combined with hydrogen peroxide and thiocyanate. The lactoperoxidase system has been used to reduce spoilage and extend the shelf-life of raw milk in countries where refrigeration may be unavailable (Asaah *et al.,* 2007). The lactoperoxidase system has been recommended to be effective in reducing the growth of *Listeria monocytogenes* in raw milk at refrigerator temperatures. However, as hydrogen peroxide and thiocyanate must be added to milk in order to activate the system to achieve antibacterial benefits, it is less likely that the lactoperoxidase system contributes significantly to control of pathogens in fresh raw milk (Leeuwen *et al.,* 2000; FAO/WHO, 2006).

Phosphatase:

This is another enzyme in milk and whose thermo liability or destruction at pasteurization temperature is used as indicator test for evaluating the effectiveness of pasteurization process (Hagstad and Hubert, 1986).

Cows milk contains 3.8% fat, 3.4% total protein, 2.7% casein, 0.7% albumin, 4.8% lactose, 0.7% mineral salts and 87.3% water (Kon, 1972).

2.2.3. Physical quality of raw Milk

Milk is a complex colloidal dispersion of fat globules and protein (casein, whey) in an aqueous solution of lactose, minerals, and other minor constituents. So, its physical characteristics are affected by several factors including the composition and processing or handling (Jay *et al.*, 2000). Physical quality of milk means the general appearance, the purity, color and flavor of the raw milk. As it is hygroscopic in its nature mainly due to the lactose content, milk attracts a number of bad smells from the immediate environment (Hagstad and Hubert, 1986). On top of this property of milk, its taste and flavor can be the function of a multiple of factors and can of course indicate the hygienic, bacterial and compositional qualities of it (Hagstad and Hubert, 1986). They can also affect consumer acceptance, which can limit the well being of the dairy business.

2.2.4. Other constituents of milk

The presence of chemical residues, poisons and contaminants in milk such as pesticides, antibiotics, mycotoxins and heavy metals is of public health concern and a cause of economic loss in the dairy industry (EHEDG, 2007). Widespread use of persistent pesticides in agriculture and mosquito eradication had resulted in the pollution of the environment and transfer of their residues to milk through feed, fodder and soil in many parts of the world (FAO/WHO, 1998). On the other hand, aflatoxins are toxic substances produced by greenish-grey moulds, *Aspergillus flavus* and *Aspergillus parasiticus* in crops, particularly peanuts and maize, provided as feeds to

dairy cows. So animals can be poisoned, if enough is consumed, and they can also cause human liver cancer, if consumed together with milk.

2.3. Factors affecting milk Quality

2.3.1. Health of the dairy herd

The herd health status can affect the overall performance of the dairy animals including the amount of milk production, reproductive efficiency and the various qualities of the milk and its products (Radostits *et al.,* 1994). It affects the bacteriological (Jay, 2000) as well as the compositional qualities of milk (Quinn *et al.,* 2002). Thus, it is very important to consider separately the effects of some important diseases in dairy cattle with respect to the microbial, nutritional and physico-chemical aspects of milk quality.

On top of their effect on milk quality and cattle productivity, different diseases of the cow have important public health implications. Of such diseases, bovine tuberculosis and bovine brucellosis are two of the major ones that can be contracted through consumption of raw milk produced by infected herd (WHO/FAO, 1953; Acha and Szyfers, 2001). Therefore, the health status of a dairy herd is the sole indicator of the safety as well as the quality of the milk produced.

Bacterial quality of milk is directly influenced by the health status of the lactating cows in general and that of the udder in particular. Increased levels of infections of the udder, as measured by the CMT score or direct somatic cells count, will increase the bacteriological load of the milk thereby causing a degradation of milk quality (Hailu, 1989; Auldist *et al.,* 1995; Murphy, 1996; Auldist and Hubble, 1998; Godefay and Bayeleyegn, 2000; Alehegn, 2004).

It has long been established that bovine tuberculosis occurs at higher prevalence rates in dairy cattle (Collins, and Grange, 1983; Cocivi, *et al.,* 1998; Goknur, 2006). Especially those dairy cattle managed under intensive management system are at greater risk of being infected because of their longer production life in a given farm environment in which case infection of even fewer number of animals can be enough to contaminate the bulk of milk produced every time from the

herd (Assegid, 1999; Eliyas, 2005; Ameni *et al.*, 2007). Dairy cattle kept in intensive farms, which may be less hygienic and more crowded, are predisposed to udder problems of both clinical and sub-clinical forms (Paul, *et al.*, 1992). On top of this, the physiological state of the cows during their gestation, parturition and lactation are the main production related factors that sets cows in to immunological stress leading to higher probability of acquiring diseases from their environment and also from their herd mates (Radostits *et al.*, 1994).

There are a number of biological functions that can be disrupted during health problems in animals kept for production purposes (Radostits *et al.*, 1994). Particularly, during the intramammary infection of dairy cows, a number of mechanisms can be disturbed; the outcome is difficult to predict and will depend on the following: a) severity of the infection; varies from very little effect to completely inhibition of milk secretion depending on the mastitis-causing organism, its virulence and resistance of the host; b) extent of the infection; which may be localized to a few alveoli or encompass all alveoli; c) alteration of the metabolic activity of milk producing cells, including reduction of milk synthesis, and interference with ionic balances, either by a reduced concentration of a galactopoietic hormones or by an increased concentration of an inhibitory hormone and an inflammatory mediators; d) interference with precursors availability for milk synthesis due to anorexia, decreased blood flow in the mammary gland or hormonal imbalance; e) disruption of epithelial integrity, by opening up paracellular pathway; f) decomposition of the milk constituents due to leukocytes' and mastitis-causing organisms' enzymes (Radostits *et al.*, 1994; Quinn *et al.*, 2002). Causing one or more of the above pathophysilogical changes, mastitis reduces both the volume and the nutritional qualities of milk (Auldist *et al.*, 1995; Auldist and Hubble, 1998; Mungobe, *et al.*, 2005).

Furthermore, microbial toxins and enzymes from damaged cells cause injury of secretory cells. Such cellular damage can produce "holes" within the mammary epithelium that can lead to changes in milk composition and short-circuit the blood-milk electrical potential in the same manner as opening of tight junctions (Linzell, and Peaker, 1991). For example, lactose, which is synthesized exclusively by mammary epithelial cells, partially leaks into blood circulation through the damaged blood-milk barrier. Simultaneously, there is an increase of the

concentrations of blood borne components in the milk of affected quarters such as serum albumin and sodium and chloride ions (McMannaman and Nevil, 2003).

It is generally accepted that during mastitis, there is an increase in milk proteins (Fernandes *et al*., 2004). This has been attributed to the influx of blood-borne proteins such as serum albumin and immunoglobulins into the milk even though coupled with a decrease in caseins (Coulona *et al*., 2002). Potassium, the most abundant mineral in milk, leaks out of milk and its concentration decreases in the milk. Conversely, the concentrations of sodium chloride in milk from cows with sub-clinical mastitis can be elevated probably due to the influx of blood constituents into the milk during infection (Nguyen *et al.,* 1998). Lactose in milk declines in affected quarters due to the damage to the alveolar epithelial cells. The more likely reason for depressed lactose concentrations is the leakage of lactose out of milk (Shennan, 2000).

Therefore, concentrations are dependent on the severity of damage to the tight junctions. Evidence for this exists in the elevated concentrations of lactose in the blood and urine of mastitic cows. The effect of mastitis on the characteristics of milk fat has not been studied as extensively as milk proteins. There are contradictory results in the literature dealing with this matter. For example, Auldist and Hubble (1998) reported a decrease in fat concentration but many other authors recorded an increase in total fat content of mastitic milk. Milk fat globule membranes are susceptible to the action of lipase enzymes, produced by leukocytes that invade the mammary gland in response to infection, resulting in breakdown of triglycerides, oxidation of fatty acids and off-flavours. It has been assumed that milk with a high SCC is more susceptible to spontaneous lipolysis (Azzar and Dimick, 1985).

The physical quality of milk is the function of a multitude of factors including the feeds given to the cows, the health status of the udder (Quinn, *et al.,* 1999), the amount and duration of bacterial action (Barbano, *et al.,* 2006) and the presence or absence of foreign materials, chemicals and impurities. Feed and health of cows directly affect the relative compositions of chemical ingredients in milk (Kazmieras, *et al.,* 2005). Due to this reason some of the physical properties of milk like the specific gravity, freezing point, electrical conductivity and other technological

properties of milk associated with requirements of processing can be lost due to poor health of the dairy animals (Tziboula, 1997).

Higher incidence and prevalence of diseases of the dairy cow particularly, mastitis in a given dairy farm results in a greater probability of having an animal treated with some chemotherapeutic agent (Quinn, *et al.*, 1999) for which purpose a wide range of antimicrobial drugs have been used in the dairy industry for decades. Treatment of lactating animals with antimicrobial drugs can lead to residues appearing in and consumed with milk (Unnikrishnan *et al.*, 2005) but official data on this aspect of milk quality in Ethiopia is unavailable for review. But it is known that the beta-lactam class of antibiotics, most often penicillin G, is the most frequently used drugs in mastitis therapy. Milk may also be co-contaminated with compounds of one of the other four major antimicrobial drug classes: the sulphonamides (e.g., sulphadiazine), tetracyclines (e.g., oxytetracycline), macrolides (e.g., erythromycin) and aminoglycosides (e.g., neomycin).

The presence of antimicrobial drug residues in milk is a public health issue as well it affects the fermentation properties of milk. Although the public health risks are difficult to define, it is accepted that antimicrobial drug residues may give allergic reactions in sensitised individuals and may have negative effects on the composition of the human intestinal flora (Paul *et al.*, 1973; Carl and Suzan, 1999).

2.3.2. Hygienic status of the farm

The production of quality milk begins with good hygienic practices. Dirty cows, soiled equipments, unhygienic parlors and dirty milker's hands all constitute an elevated bacterial level in the bulk tank. Several research results have shown that milk produced and handled under hygienic conditions can be expected to have colony counts of less than 2×10^4/ml before pasteurization while milk produced unhygienically can have bacterial load as large as millions and billions of bacteria per milliliter (Hailu, 1989; Murphy, 1996; Slagihuis, 1996; Godefay and Bayeleyegn, 2000; Alehegn, 2004; Chaye *et al*., 2004; Donkor *et al.*, 2007).

2.3.3 Feed of the dairy herd

Manipulating a ration of dairy cows can change milk composition (Grummer, 1991; Castillo, *et al.,* 2003). Decreasing the amount of forage in relation to the amount of concentrate will quickly decrease milk fat with only a variable and slight increase in milk protein percent (Mantysaari, 2003). Concentrates that are primarily composed of rapidly digested grains (e.g.: barley) will have more effect on depressing fat and increasing protein than slowly digested grains (Charles, 1998). Generally, improvements in feed protein quality will show an increase in milk yield than milk protein (Mantysaari, 2003).

2.4. Milk quality control concepts and methods

The term "quality" refers to the expectations clients have regarding a certain service or product. Implicitly it refers to both the technical features of the product, the production process from which the product originates and the perception that the client has about both. The dairy industry is highly susceptible for incidents affecting the public image of their products. It means that all efforts have to be directed toward the quality features of the product and the production processes that have direct association with the consumer concern (Nordhuizen, 2003).

There are different concepts for quality control: good manufacturing practice (GMP), international standardization office (ISO) systems, hazard analysis critical control points (HACCP), total quality management (TQM). GMP refers to rather an attitude or mentality oriented approach than a true quality assurance programme. The HACCP concept is the best choice, if a quality control programme should be designed for dairy farms, particularly because it is highly farm-specific, easy to link up with operational management, relatively low in cost, both product and process oriented, and not requiring much labor. ISO is very laborious and costly as well as far too non-specific to make it truly workable for a dairy farmer. In any case, a sound quality attitude of farmers and others involved is needed before one should even think about introducing HACCP or ISO. The concept of TQM according to Evans and Lindsay (1996) can be considered as a merger of GMP and HACCP concepts (Noordhiuze, 2003a; Nordhuizen, 2003b).

The first step in controlling the quality of the milk supply is inspection of farms, dairies and food stores to see that this very perishable foodstuff is produced and handled under reasonably cleanly conditions (Jay *et al.*, 2000). The benchmark for quality of raw milk is the standard plate count of less than 10,000cfu/ml. Most high quality dairy farms routinely achieve bacteria counts of 5,000/ml or less (Kurwijila *et al.*, 1992). It is possible to regularly achieve low bacteria counts and if these levels are not being attained, take a hard look at each and every component involved, especially the ones noted as the main sources of bacterial contamination to the milk like the prevalence status of mastitis in the herd and the hygienic and sanitary conditions of the milk production process (Kivaria *et al.*, 2004; Mendez *et al.*, 2006). The primary producers of milk, the dairy farms, are not formally engaged in quality assurance programmes mainly due to the difficulty in setting standards that quality control at dairy farm level goes beyond the quality control of the product milk alone (Noordhuizen and Welpelo, 1996).

3. MATERIALS AND METHODS

3.1 Study area

This study was carried out in Addis Ababa city, which has 10 administrative sub-cities. It is located in the central highlands of Ethiopia, covering an area of 530km^2 with an elevation ranging from 2000-2800 m.a.s.l. The air temperature is fairly constant throughout the year, with variations between 20 to 25 ^{0}C during the day, and between 7 and 11 ^{0}C at night. Average rainfall is 1200 mm per year, with the major rains occurring between July and September. Population density reaches 632 inhabitants/ha in the slum areas and 5 inhabitants/ha in the rural Addis Ababa. Due to this higher population density land is a very scarce resource that little is available for the growing number and size of intensive dairy farming while the sector requires considerable size of land which is in short (Ketema, and Tsehaiy, 1995).

Addis Ababa is a city where various forms of urban agricultural activities are under taken usually as a side line business and a leisure time duty. The dairy production system is part of this urban agricultural system characterized by intensive production operations in which dairy animals are kept in-doors at zero grazing conditions. The greatest majority of the peri-urban and intra-urban dairy producers do produce intensively for the sole purpose of selling the milk directly to consumers and retailers (Ketema and Tsehay, 1995). Particularly in response to the growing demand of foods of animal origin like milk that has special type of demand characterized by price- inelasticity, large numbers of large-scale dairy farms are being established (Mohamed *et al.,* 2003).

3.2. Study animals

The present research was conducted on a total 1031 animals in 31 dairy farms out of which 400 are females at their various stages of lactation. Depending on the type and prevalence of the diseases of interest variable sample sizes were considered. Thus the study of mastitis, bacteriological quality of bulk milk and herd status for brucellosis were undertaken on 400 cows

and the bulk milk in 31 farms, while 26 farms with a total herd size of 671 animals were studied for the prevalence of tuberculosis.

3.3 Study design

A cross sectional study design was conducted from October 2007 to April 2008 in order to investigate the microbiological quality of milk and the possible public health hazards due to brucellosis and tuberculosis from dairy farms in Addis Ababa.

3.4 Sampling procedure and sample size determination

The One stage cluster sampling was used whereby farms were selected randomly from a sampling frame provided by Shola Regional Veterinary Laboratory and all animals in the selected farms were sampled. The total number of the farms to be sampled was determined based on the standard formula for a single stage cluster sampling given by Thrusfield (2005).

$$G = \frac{(1.96)^2((nVc + (P_{exp})(1- P_{exp}))}{nd^2}$$

Where: G = the number of clusters/farms to be sampled

n= average cluster size

d = degree of desired absolute precision

P = expected prevalence of the disease

Vc = within cluster variance

In the Addis Ababa area it has been reported that bovine tuberculosis, mastitis and brucellosis had prevalence rates of 52.4% by Elias (2005), 46.6% by Mungobe (2001) and 64.3% by Yilikal (1998), respectively. Of these figures sample size calculation was done using the 52.4% prevalence for tuberculosis. The within-cluster variance was estimated to be 0.0001 while the

average herd size was estimated to be 20 (Elias, 2005). Calculations yielded minimum number of farms to be 20. Accordingly, the total number of the animals required in this study was 400. The sample size for the prevalence estimation of the other diseases of interest falls below that required for tuberculosis which showed maximum variance as it has a prevalence near to 50%. But, in an effort to keep precision well above the desired level (Thrusfield, 2002) as well as expecting lower response rate of farmer (<75%), 35 farms were initially contacted from which 31 of them became willing to participate in the study. For the purpose of investigating the bacteriological quality of milk, bulk milk samples from each of the 31 farms were collected 5 times that made a total of 155 bulk milk samples.

3.5. Data collection methods

3.5.1. Questionnaire Survey

Semi-structured questionnaires were prepared, pre-tested and amended as deemed necessary and one visit interview was carried out on selected farm households in order to gather information on potential risk factors related to the health of cows and the quality of the milk produced in each farm. Such factors as the housing conditions, hygienic status, ventilation, udder cleaning and milking practices were given due emphasis in the course of the questionnaire. The age, sex and educational level of the farm owners was also recorded (Annex 3).

3.5.2. Standard plate count

Bulk milk samples were collected right after milking from large containers into which all the milk from all the milking cows in the farm was pooled together. The samples were transported to the Shola Regional Veterinary Laboratory in cold boxes supplied with reusable ice packs. Microbiological culturing of these samples was done within 24 hours of collection from the farm. SPC was performed according to ES-ISO (Ethiopian Standards-International Standards Organization (2001)) procedures.

When plates contained less than 25 colonies, the results were read as less than $2.5x10^2$ (in 1:10 dilution). If more than 250 colonies developed on the highest dilution plate, the count was

recorded as more than 250 times the reciprocal of the dilution. When computing the standard plate counts (SPC), only the first two significant digits were reported to avoid creating a fictions impression of precision and accuracy. When making the conversion, the second digit was rounded off to the next highest number when the third digit was greater than equal to six and rounded down when the third digit was less than equal to 4. When the third digit is 5, the second digit was rounded up if the second digit was odd and rounded down when the second digit was even (ES-ISO, 2000).

3.5.3. Diagnosis of mastitis

Clinical mastitis was diagnosed on the basis of manifestation of visible signs of inflammation. A quarter, which was warm and swollen and had pain upon palpation was considered to have acute clinical mastitis, while misshaped, atrophied, hard and fibrotic quarter was considered to have chronic mastitis (IDF, 1987).

The California Mastitis Test (CMT) was carried out following the procedure described by Quinn *et al.* (1994) for sub-clinical. Briefly, a drop of the CMT reagent was put on the 4 cups of the CMT pad into which equal amount of milk from the respective quarters of the cow was added and gently mixed by rotating the pad in a horizontal plane. The test result was interpreted based on the thickness of the gel formed by CMT reagent and milk mixture and scored as negative (o), trace (T), + (weak positive), ++ (distinctive positive), and +++ (strong positive). Quarters with CMT score of + or above were judged as positive. Cows were considered positive for CMT, when at least one quarter turned out to be positive for CMT. A herd was considered positive for CMT, when at least one cow in a herd was tested positive with CMT. The total number of blind teats as well as those with clinical infection was subtracted from the total number of teats and the difference was used to calculate the prevalence of sub-clinical mastitis.

3.5.4. Comparative intradermal tuberculin test

The standard method for detection of bovine tuberculosis which is the comparative intradermal tuberculin test was carried out according to OIE (2004). It was performed at the mid side of the

neck at which site the hair is clipped off, the skin disinfected and skin thickness was measured using a caliper and the pre-PPD injection. Then followed the intradermal injection of the purified protein derivatives PPD of the two organisms *Mycobacterium bovis and Mycobacterium avium.* Two sites at the middle of the neck 12.5 cm apart were shaved on the same side of neck, and skin fold was measured with a caliper. Then 0.2 ml of bovine PPD (Bovitubal, strain AN5, Bioveta, Czech Republic) and 0.1 ml (28,000 TU/mL) of avian PPD (Avitubal, strain D4ER, Bioveta, Czech Republic) were injected intradermally. The sites were examined and skin thickness was measured 72 hours later. Interpretation of the result was made according to OIE (2004).

3.5.5. Milk ring test for bovine brucellosis

Milk ring test was used to study brucellosis in the dairy farms of Addis Ababa following the procedure of OIE (2004). Stained brucella antigen (Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore, India) at a volume of 30-50µl was added to 1-2ml of whole milk obtained from bulk tank. The antigen-milk mixture was then incubated for 1 hour at 37 ^{0}C and formation of a dark blue ring above a white milk column as a result of antigen-antibody interaction was recorded as positive for brucellosis (OIE, 2004)

3.7. Statistical analysis

Data was entered in Microsoft Excel program. Descriptive statistics was used to summarize the collected data. Associations of prevalence of tuberculosis, brucellosis and mastitis with potential risk factors were analyzed using univariate and multivariate logistic regression methods. Comparison of means of bacterial load was done by univariate ANOVA. SPSS (release 11.05, 2002) was used to analyze the data.

4. RESULTS

4.1. Description of study farms

The results of descriptive statistics showed that about 45.2% of the household heads were retired (45.2%) and the proportion of male (48.4%) and female (51.6%) decision makers were comparable. Most of the farmers attended only elementary school (48.4%) while some attended secondary (35.5%) and tertiary level of education (16.1%). The average dairy cattle herd size was 33.2 heads from which the majority was made of lactating cows (53.52%) followed by almost equal proportions of dry cows, heifers and calves (13.1-13.49%) (Table 4).

Table 4. Dairy cattle and herd size and composition per farm in the study area

Cattle types	N	Mean (SE)	Proportion
Lactating cows	550	17.77 (0.57)	53.52
Dry cows	139	4.48 (0.34)	13.49
Heifers	135	4.35 (0.09)	13.10
Calves	135	4.35 (0.11)	13.10
Bulls	35	1.30 (0.12)	3.92
Herd size	1028	33.2 (0.03)	100.00

All the selected dairy farms did not receive any training on modern husbandry practices and did not keep records at all or their records are fragmented and less informative. Their herds are not diagnosed for major animal diseases like tuberculosis, brucellosis and mastitis within six months of time and even if diagnosed once up on a time, they are not informed about the results. The majority of the farms (90.3%, n =28) surveyed were poorly drained, less ventilated and crowded. None of them had separate parlors for milking cows. It was observed that potable water was in short in 12 of the farms (38.7%). About 58.1% of the farms were cleaning the udder and the proportion of farms using soap and common towel to clean udder was 58.1% and 93.5%, respectively.

4.2. Results of bacteriological culturing of milk

The bacterial counts in colony forming units per ml of milk samples were first transformed into the natural logarithms in order to make the distribution normal. The mean, standard error and 95% confidence intervals are presented in Table 5. The mean SPC of milk collected from the sampled farms ranged from 5.39-5.50 log cfu/ml. The results of univariate ANOVA indicated that there was no significant variation observed in the mean log cfu/ml of SPC between the different categories of hygienic practices, availability of potable water and herd size ($p>0.05$).

Table 5. Results of univariate analysis of variance on effects of risk factors on SPC

Factors	Category	N	Mean (SE) of SPC	95% Confidence Interval	P-value
Hygienic practices	Good	45	5.45 (0.09)	5.25 - 5.64	0.742
	Bad	110	5.49 (0.05)	5.38 - 5.60	
Availability of potable water	Available	75	5.39 (0.07)	5.25 - 5.55	0.112
	Not available	80	5.50 (0.06)	5.43 - 5.68	
Herd size	<40	105	5.48 (0.06)	5.34 – 5.49	0.794
	>40	50	5.49 (0.08)	5.34 – 5.64	

4.3. Prevalence of clinical and sub-clinical mastitis

The results of CMT for subclinical mastitis are presented in Table 6. The overall quarter level prevalence of subclinical mastitis was 83.03% (n=1042) from which 34.5%, 20.96%, 17.69% and 9.88% of the quarters showed strong, distinct, weak and trace positive reactions. The remaining 16.97% of the quarters showed negative reaction to CMT.

Table 6. The frequencies of the categories of CMT scores in studied quarters

CMT Score	Frequency	Relative proportion (%)	Cumulative Frequency	Cumulative proportion (%)
Strong positive(+3)	433	34.50	433	34.50
Distinct positive(+2)	263	20.96	696	55.46
Weak positive (+1)	222	17.69	918	73.15
Trace	124	9.88	1042	83.03
Negative(0)	213	16.97	1255	100
Total number of teats	1255	100		

The prevalence of subclinical mastitis at cow and herd level was 81.8% (n=327) and 100% (n=31), respectively. The prevalence of clinical mastitis at quarter, cow and herd was 3.4% (n=52), 6.5% (n=26) and 38.7% (n=12), respectively. Of the total 1600 teats inspected in the 400 cows the proportion of cows with at least one blind teat was 7% (n=28) while the overall frequency of teat blindness was 2.81% (n=45) of the diagnosed quarters. About 3.75% (n=15), 2.25 (n=9) and 1% (n=4) of the cows had one, two and three blind teats, respectively.

The results of univariate logistic regression analysis showed that parity ($p<0.05$), udder cleanliness ($p<0.05$), farm hygiene ($p<0.05$), stage of lactation ($p<0.05$) and herd size ($p<0.001$) had significant association with quarter level clinical mastitis. The highest prevalence of quarter level clinical mastitis was observed in early (1.6%) and late parities (1.5%), in cows with poor udder cleanliness (2.13%), in farms with poor hygiene (2.0%), in late lactation stage (1.8%) and in farms with herd size ranging from 26 to 40 (1.56) (Table 7).

Table 7. Associations of potential risk factors with clinical and sub-clinical mastitis

Risk factors	Categories	Clinical mastitis (N=1600 quarters)			Subclinical mastitis (N=1255 quarters)		
		N	Prevalence	P-value	N	Prevalence	P-value
Parity	≤ 2	16	1.60	0.040	300	23.9	0.017
	3 & 4	12	0.75		398	31.7	
	> 5	24	1.50		557	44.4	
Udder cleanliness	Good	18	1.13	0.013	349	27.8	0.485
	Poor	34	2.13		906	72.1	
Farm hygiene	Good	20	1.25	0.026	416	33.1	0.355
	Poor	32	2.00		839	66.8	
Stage of lactation	Early	9	0.56	0.020	262	20.9	0.004
	Middle	14	0.89		372	29.6	
	End	29	1.81		621	49.5	
Herd size	10-25	21	1.31	0.000	404	32.2	0.005
	26-40	25	1.56		428	34.1	
	>40	6	0.38		423	33.7	

N=Number of Observations

On the other hand, the prevalence of subclinical mastitis at quarter was associated significantly only with parity number ($p<0.05$), stage of lactation ($p<0.01$) and herd size ($p<0.01$). The prevalence of subclinical mastitis significantly increased with parity number and stages of lactation. The highest quarter level prevalence of subclinical mastitis was observed in the herd size ranging from 26-40 (Table 7).

4.4. Prevalence of bovine tuberculosis

The overall prevalence of bovine tuberculosis was 62.66% when only the distinctive positive animals were included but the prevalence went up to 68.41% when inconclusive reactors were

also included. The number of positive reactors and prevalence of bovine tuberculosis are presented in Table 8.

Table 8. Results of the comparative intradermal tuberculin test of bovine tuberculosis

Tuberculin reaction	Number of positive reactors	Prevalence
Positive	396	62.56
Inconclusive	37	5.85
Negative	200	31.60

The analysis of prevalence in specific risk groups indicated that the disease occurs in significantly higher rates in farms with bad ventilation ($p<0.05$), in cows with 3 and 4 parity number ($p<0.05$) and in farms with larger herd size ($p<0.05$). In the present study, age, sex and stage of lactation had no significant association with the prevalence of bovine tuberculosis ($p>0.05$) (Table 9).

Table 9. Association of risk factors to bovine tuberculosis

Risk factors	Categories	Bovine tuberculosis		
		N	Prevalence	P-value
Age (N=633)	>0.5<2 years	123	54.9	0.427
	2-4 years	263	66.1	
	>4 years	247	67.2	
Sex (N=633)	Male	66	54.6	0.358
	Female	567	63.5	
Parity (N=631)	≤ 2	382	62.3	0.021
	3 and 4	209	65.6	
	> 5	40	52.5	
Ventilation (N=633)	Good	146	53.1	0.011
	Bad	487	65.7	
Stage of lactation (N=524)	Early	69	66.7	0.896
	Middle	183	63.4	
	End	272	62.5	
Herd size (N=633)	10-25	224	54.9	0.026
	26-40	171	66.1	
	>40	238	67.2	

The results of multivariate logistic regression showed that infection of the dairy cows by bovine tuberculosis was explained more by herd size and the ventilation status. Parity did not have significant effect on bovine tuberculosis in the multivariate logistic regression.

4.5. Prevalence of bovine brucellosis

The prevalence of bovine brucellosis based on milk ring test in 31 bulk milk samples was 12.9% (n=4). There was significant association between the herd level prevalence of bovine brucellosis and the occurrence of abortion (6.45%).

5. DISCUSSION

In this study, the majority of the farms (90.3%, n =28) surveyed were poorly drained, less ventilated and crowded. It was observed that potable water was in short in 12 of the farms (38.7%) and the proportion of farms using soap and common towel to clean udder was 58.1% and 93.5%, respectively. This could facilitate the spread of micro organisms of environmental and faecal origin and could also be responsible to contaminate the milk (Fox and Gay, 1993; Quinn *et al.,* 2002). Separate milking parlors were not available in the studied farms which could increase the risk of contamination of milk by microbes of the faecal and environmental origin (Chaye *et al.,* 2004).

All the farms surveyed were in the intra urban agro-ecology and they exercised zero grazing and hence all animals were in total confinement. It has been demonstrated that dairy cows maintained at zero grazing are vulnerable to deficiency of vitamins A, E and Se (Eriskin *et al.,* 1987) and are susceptible to infection by mastitis causing pathogens because of weaker epithelial cell integrity and possible degeneration.

The proportions of blind teats (3.4%) obtained in the present study are in closer agreement with 3.72% reported by Mungube (2001) and 3.8% reported by Kassa *et al.* (1999). But this result is slightly higher than the report of Getahun *et al*. (2008) (2.1%). This difference is attributed to the difference both in the production system as well as the associated higher incidence of clinical and subclinical mastitis which were lower in the herds of the small holder farmers studied by Getahun *et al*. (2008).

The study on the bacterial quality of milk based on the SPC indicated that the mean SPC was 5.52 log cfu/ml in conditions of good farm hygiene and 5.47 log cfu/ml in farms with poor hygienic status. The overall mean SPC was 5.49 log cfu/ml. This is not of any statistical significance but the microbial load in more than half of the samples (54.2%) was beyond the recommended level of 300,000 cfu/ml. And 16 (10.3%) of samples had bacterial counts greater than the order of 10^6 cfu/ml. Lack of statistically significant difference in mean SPC in different levels of farm hygiene could be associated with the involvement of many interacting factors

affecting milk bacterial quality (Godefay and Molla, 2000; Alehegn, 2004; Dessalegn, 2005). In many countries similar results have been reported by Chaye *et al.* (2004) in Malaysia, Elmagli *et al.* (2006) in Sudan, Afif *et al.* (2007) in Morocco and Donkor *et al.* (2007) in Ghana. Milk with high bacterial load is likely to endanger the health of the general public as some pathogens like S*taphlocacus aureus, E. coli, Streptococci, Salmonela* and *Listeria* could make up the bacterial population (Alehegn, 2004, Desalegn, 2005). In addition, shorter shelf life and deteriorated nutritional quality has been attributed to the higher bacterial counts (Quinn *et al.,* 2002).

Increased bacterial count in bulk milk samples as the function of the farm hygiene and the milking sanitation has been shown in studies by Chaye *et al*. (2004) and Alehegn (2004). However, in this study there was no significant association of milk bacterial load and the different categories of hygienic practices, availability of potable water and herd size ($p>0.05$).

The prevalence of subclinical and clinical mastitis at cow in this study was 81.8% (n=327) and 6.5%. The finding on cow level clinical mastitis is close to that of Bishi (1998) (5.7%) and Mungobe (2001) (6.6%) in the same study area. But the results of the prevalence of sub-clinical mastitis in the present study (81.8%) has been significantly higher than reports from previous studies indicating 40%, 34.3% and 46.6% by Kassa *et al*. (1999), Bishi (1998) and Mungube (2001), respectively. The explanation to this remarkable difference in the prevalence of sub-clinical mastitis could be the types of the farming systems as well as the scale of operation which were different in the different studies. In the current study only large scale farms with exclusively intensive operation has been studied.

Risk factor analysis for both clinical and sub-clinical mastitis indicated that cows in early parity, with poor udder hygiene, in late stages of lactation and kept in farms with poor hygienic practices and larger herd size were at greater risk of clinical mastitis. The same trend of association was observed between subclinical mastitis and late stage of lactation and larger herd size in this study. This type of association has been observed in other similar studies also (Mungube, 2001; Bishi, 1998, Kassa *et al.,* 1999). It has been established that efficiency of disease transmission is higher in larger herd sizes in complete confinement and crowded premises (Seifert, 1996). Confined dairy operation would also mean poor hygiene unless much time and labor is invested to keep

cows and their premises clean at all times. Thus, pathogens of environmental origin like *E. coli* and those which can survive both in the cow's udder as well as the farming premise like *Staphylococcus aureus* would be maintained nicely (Radostits *et al.*, 1994; Quinn *et al.*, 2002).

In the present study, the prevalence of bovine tuberculosis was found to be 62.66% and the disease was so remarkably prevalent in herds with large size, in poorly ventilated condition and in cows with 3 and 4 parity numbers. Animals of different age, sex and stage of lactation were at equal risks of the diseases. Ameni *et al.* (2007a) have reported a prevalence of 48% near Addis Ababa which is lower than the finding of this study. But factors which predisposed cattle to bovine tuberculosis in the present study have been pointed out in the studies of both Ameni *et al.* (2007a) and Assegid *et al.* (2000). The overall prevalence of bovine tuberculosis obtained in this study 62.66% is comparable with the findings of Elias (2005) (l58.7%-77.8%) in farms with more than 20 heads per herd.

The increasing risk of tuberculosis in dairy cattle as the herd size is increased in consequence to intensification is consistent with the reports in the same study area by Asseged *et al.* (2000). Higher prevalence of bovine tuberculosis in larger herds might be due to the fact that the risk of introducing tuberculosis infection into a negative herd by an individual animal may increase with herd size (Siefert, 1996). Significant association of prevalence of bovine tuberculosis with age as well as the lactation stage of the cows was reported by Assegid *et al.* (2000), Elias (2005) and Ameni *et al.* (2007) which was not the case in this study. Increased herd size and poor ventilations, which were found to be associated with high prevalence of bovine tuberculosis, predispose animals to the disease more than other factors. Both of these conditions are the results of intensification in which dairy cattle are aggregated in larger numbers but smaller spaces. Such suffocated environment is in favor of effective aerosol transmission of *Mycobacterium bovis* (Radostits *et al.*, 1994; Quinn *et al.*, 2002).Moreover adult animals at their good production life with 2 and 3 parities were at greater risk of infection (65.6%) in the present study as compared to animals of higher parities (53.1%). The intra dermal tuberculin test used depends on how well the animals' bodies respond to the antigen. In cows with older infections, in adult animals with immunocompromization and in very young calves where the immune system is not developed yet, the test is less likely to detect a case (Tizard, 1998).

Studies on the prevalence of bovine brucellosis in 31 farms bulk milk samples using MRT indicated a 12.9% herd prevalence of antibodies to *Brucella abortus* and the result is in exact agreement to the reports of Yilkal (1998) who reported an overall herd prevalence rate of 12.5% in the intra- urban dairy farms of the same area. The same author has observed that the sensitivity of the MRT was 57.1% compared to CFT which would mean that the current finding on the magnitude of the disease based on MRT would have under estimated the prevalence of the disease. Yet, OIE (2004) have put this test as excellent screening test to identify herds with at least one infected lactating cow by Brucella organisms, particularly with herd sizes less than 100 lactating cows. Despite the poor record of data in all the farms, herd history of abortion have been found to associate with the prevalence of bovine brucellosis indicating the possible negative impact of the disease in the productivity of the dairy animals. Owing to the fact that the large scale farms surveyed in the current study supply milk in raw to the public, there is an implication that the disease could also have jeopardized the public health.

6. CONCLUSSIONS AND RECOMMENDATIONS

Based on the findings of this study it is possible to conclude that raw milk produced by the intensive dairy farms in Addis Ababa do not comply with the internationally accepted level of bacterial load of 300,000 cfu/ml of milk. The high bacterial load was invariably common to all studied farms.

The prevalence of clinical mastitis in the studied farms was moderate but that of subclinical mastitis and bovine tuberculosis was generally high. The level of herd infection by brucellosis was also considerable. Parity, udder cleanness, farm hygiene, stage of lactation and herd size were determinants of the occurrence of clinical mastitis while only parity, stage of lactation and herd size were associated with the occurrence of subclinical mastitis. The prevalence rate of bovine tuberculosis was affected by parity, level of ventilation of farms and herd size.

From the overall findings it is evident that raw milk being marketed by the farms in the city is no way safe to the health of the general public mainly on account of the high prevalence of zoonotic diseases including tuberculosis and brucellosis and higher bacteriological content.

Based on the conclusions made from this study the following recommendations are forwarded:

- National milk quality standards and regulations demarcating the minimum operational environments of the routine husbandry practices and health requirements of dairy animals should be instituted;
- In addition, the regulatory bodies mandated to safeguard public health, animal health and food safety should join hands in an effort to help dairy producers to strive for quality milk production through legislative enforcement, incentives per quality and through extension services;
- Well designed and coordinated control programs for bovine tuberculosis, brucellosis and mastitis in dairy farms at a national level in general and in the Addis Ababa intra-urban production sub-system in particular should be initiated by the Government;

- Awareness should be created among producers and consumers of milk and milk products as to the risks of consumption of raw milk and milk products;
- It is very evident that milk produced in such conditions is of poor nutritional and keeping quality, thus further studies should be carried out to determine the effect of the specific diseases on the public health, productivity of the dairy herds and on the nutrient contents of milk.

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8. ANNEXES

Annex 1. Interpretation and Scoring of the California Mastitis Test

Score	Meaning	Description of the visible reactions
N	Negative	Mixture remains liquid, homogeneous, with no evidence of thickening.
T	Trace	The slight thickening that forms is seen best by tipping the paddle back and forth and observing the mixture as it flows over the bottom of the cup. Trace reactions tend to disappear with continued rotation of the paddle. Read at 10 seconds.
1	Weak Positive	A distinct thickening of the liquid forms, but there is no tendency toward a gel formation. With some milk, the thickening may disappear after prolonged rotation of the paddle (20 seconds or more). Read at 10 seconds.
2	Distinct Positive	Mixture thickens immediately, and a gel formation is suggested. As the mixture is swirled, it tends to move in toward the center, exposing the bottom of the outer edge of the cup. When the motion is stopped, the mixture level out and covers the bottom of the cup. Read at 10 seconds.
3	Strong Positive	A gel is formed, which causes the surface of the mixture to become elevated like a partially fried egg. There is usually a central peak that remains projecting above the main mass, even after the rotation of the paddle is stopped.

Annex 2. Pathogenic bacteria of public health significance from raw milk

Pathogenic bacteria	Remark
Mycobacterium bovis / Mycobacterium tuberculosis	Tuberculosis
Brucella abortus / Brucella melitensis	Brucellosis
Coxiella brunetii	Q-fever
Staphylcoccus aureus	Enterotoxin
Escherchia coli	Some serotypes pathogenic to man,
Salmonella	Salmonellosis
Streptococcus agalactae	Pathogenicity for man uncertain
Leptospira spp	Other source (feces, poor silage)
Listeria monocytogenes	Other source (feces, poor silage)
Bacillus cereus	Survive pasteurization, other sources
Clostridium perfringens	Survive pasteurization, other sources

Source: Slaghuis (1996)

Annex 3. Questionnaire and farm observation sheet

I Questionnaire format- general aspects

1. Physical address_____

2. Farm owner _____sex_____age_____

3. Education : elementary_____ Secondary_____ College_____

1. Total Herd size_____

2. Lactating cows _____Dry cows_____ Heifers_____Bulls _____calves_____
3. Breeds_____ local _____ crosses_____
4. Milk production_____it/day. Amount sold_____lts/day.
5. Markets used, name_____
6. Number of employed workers_____
7. Average salary of employees_____
8. Health checkup Yes_____No_____
9. if yes which diseases, Name_____ _____ _____
10. Have you received training Yes_____No_____
11. If yes on what topic of dairy husbandry? Name_____
12. Name the main problems affecting your productivity _____

_____

II Farm hygiene and milking procedures observation

Farm hygiene and drainage Good _____ Bad_____

Floor type Soil_____concrite_____

Walls and Roofs Roofed _____ Open_____

Udder cleanliness clean_____ Dirty_____

Detergents used Yes_____ No_____

Pre milking udder washing serious_____indulgence _____

Separate udder towels _____ shared towels_____

Premilking calf suckling Yes_____No_____

Post milking teat dipping Yes_____No_____

If Yes, what chemical used_____

Availability of potable water

Available_____ Not Available_____

Farms ventilation: Suffcated_____Airated_____

Annex 4. Procedure of standard plate count

1. One milliliter of milk sample was diluted in 9 mL of sterilized peptone water at 0.1% concentration
2. Dilutions proceeded to obtain a 10^{-5} dilution and at each step of dilution the milk suspension was mixed using an electronic shaker Vortex shaker.
3. One milliliter of each dilution was transferred to sterilized Petri dishes using use-and-throw sterile pipettes.
4. Each dilution was cultured in duplicate and 12–15 mL of molten culture medium (Plate Count Agar, OXOID) at a temperature of 45-50 ^{0}C was poured into the dishes (Pour plate method) and was well mixed using automatic shaker.
5. Prepared dishes were inverted and placed in the incubator at 30°C for 72 h. Colonies were counted with an automatic colony counter (Q Count; Spiral Biotech, Inc.).
6. The total number of cfu/ml was calculated using the following formula:

$$N = \frac{\sum colonies}{\left[(1xn1) + (0.1xn2)d\right]}$$ Where: N = number of colonies per milliliter of milk,

$\sum$C = sum of colonies on plates counted, n_1= number of plates on lower dilution counted,

n_2 = number of plates in next higher dilution counted and d = dilution from which the first counts are obtained.

Annex 5. Clinical Examination Sheet

Animal identification_____ Date of examination _____

Owner name_____ farm code/Id_____

Breed_____age _____ parity number_____

Physical clinical examination.

Local examination a) Discharge from the udder_____

b) Leakage of teats_____

c) Palpation of teats and quarters_____

d) Udder skin temperature_____

e) Swelling observations_____

f) Patency of teats_____

g) Gross milk appearance_____

Watery_____ blood tinged_____ clots_____yellowish_____

Systemic examination

- rectal temperature,_____
- udder temperature_____
- appetite off feed_____ inappetance_____
- General

  Remark_____