ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

BACTERIOLOGICAL QUALITY OF BOVINE MILK IN SMALL HOLLDER DAIRY FARMS IN DEBRE ZEIT, ETHIOPIA

BY

ALEHEGNE WUBETE YIRSAW

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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 Veterinary Medicine

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I the undersigned, declare that the thesis is my original work and has not been presented for a degree in any University.

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ABBREVIATIONS

CFU	Colony forming unit
Cfu/ml	Colony forming unit per ml
CMT	Californian mastitis test
CNS	Coagulase negative *Staphylococcus*
EU	European Union
HTST	High temperature short time
IDF	International dairy federation
IMC	International Mastitis Council
ISO	International standard organization
Log_{10}	Logarism in base ten
LTLT	Low temperature long time
MPN	Most probable number
SCC	Somatic cell count
SPC	Standard plate count
TAPC	Total aerobic plate count
USPHS	United States of Public Health Service
UHT	Ultra heat temperature

ABSTRACT

The critical control points in milk handling, determination of bacterial load of milk at each critical control points and farm water, isolation and identification of bacterial pathogens in milk were determined from September 2003 to March 2004. A total of 178 raw milk samples from various critical points, and 77 farm water samples were taken three times from 27 small holder dairy farms of Adaa-Liben district dairy and dairy products producers and marketing co-operative society. Pasteurized milk samples (n=100) were taken from Mama milk processing plant. Specific gravity, alcohol and Californian mastitis tests were used as screening tests, and total aerobic plate counts (TAPC) and coliform counts from milk and the most probable number (MPN) of coliform counts from farm water were conducted. Isolation and identification of the bacteria in the milk was conducted following standard methods.

The specific gravity values of milk from the dairy owner's were in the range of 1.025 to 1.029. The normal range being 1.026 to 1.032 at 20 ^{o}C. Nine percent of the samples had values below 1.026, an indication of some adultration with addition of water. All the milk samples tested were alcohol test negative. Pooled milk was taken from 25 dairy farms and the CMT results showed that 76 % of the total samples were CMT negative and the remaining 24 % were positive for CMT, indicating the presence of mastitis.

The mean total aerobic plate counts of raw milk samples analyzed were $2.10x10^5$ (udder), $1.58x10^7$ (bucket), $1.50x10^8$ (storage container), and $5.67x10^9$ cfu/ml upon arrival at processing plant. The mean coliform counts were $4.84x10^3$ (udder), $1.37x10^5$ (bucket), $1.67x10^6$ (storage container), and $1.26x10^7$ cfu/ml upon arrival at processing plant. The increment of the TAPC and coliform counts at each critical control points were observed statistically significant ($P < 0.001$) for both counts. There was no significant variation between milk collection centers and the interactions between milk collection centers and critical control points for TAPC and coliform counts ($P > 0.1$). According to international standards of raw milk quality both the TAPC and coliform counts have values above the upper limits set. These include 10.4% of the raw milk samples from udder, 45.4% of raw milk samples from milking bucket and 100% of raw milk samples from storage containers and

upon arrival at processing plant for TAPC.and 37.7% of the raw milk samples from udder, 96.1% of raw milk samples from milking bucket and 100% of raw milk from storage container and upon arrival at processing plant for coliform counts. Analysis of water samples from the farms revealed that 54.6% were poor (non potable) quality.
Pasteurized milk in this study had TAPC and coliform counts ranging from $2.65x10^3$ to $7.2x10^5$ and 0 to $7.5x10^3$ cfu/ml, respectively. Based on the international standards, 7% and 27% of pasteurized milk fall below the standards set for TAPC and coliform counts, respectively.

In the course of this study the frequent bacterial pathogens isolated from raw milk samples taken from the udder include: *Staphylococcus aureus, Staphylococcus intermidus, Staphylococcus epidermidus, Staphylococcus chromogenes, Streptococcus agalactae, Streptococcus uberis, Streptococcus bovis, Corynebacterium bovis, Corynebacterium ulcerans* and *Pseudomonas aeroginosa*. Similarly, samples from milking bucket, storage container and upon arrival at processing plant were additionally contaminated with bacterial pathogens such as *Micrococcus, Rhodococcus* spp, *Enterococcus fecalis*, *Bacillus* spp, *Escherchia coli*, *Pseudomonas aeroginosa*, *Enterobacter aerogenes*, *Enterobacter aglomerans*, *Klebsiella pneumonia* and *Citrobacter freundi*. *Streptococcus pyogenes* and *Enterobacter aerogenes*. Bacteria of public health significance isolated were *Staphylococcus aureus, Streptococcus agalactae, Corynebacterium ulcerans, Corynebacterium haemolyticum, Streptococcus pyogenes* and *Escherichia. coli.*

Bacterial pathogens such as *Staphylococcus intermidus, Staphylococcus epidermidus, Staphylococcus chromogenes, Streptococcus pyogenes Enterococcus faecium, Corynebacterium haemolyticum, Corynebacterium ulcerans and Klebsiella oxytoca* which are normally eliminated by efficient pasteurization, were still present in the pasteurized milk samples.

The present study showed that:

- TAPC and coliform counts of raw milk were increased at all critical points from udder to upon arrival at processing plant significantly. However, the counts decreased to lower level after pasteurization.
- Most probable number (MPN) of coliform counts from farm water samples showed 55.6% were of non-potable quality

- The number and types of isolated bacteria increased after the milk left the udder to upon arrival at processing plant due to exogenous contamination sources.
- The health of dairy herd, milking and storage conditions, unclean milk equipment, frequent transferring of milk into different containers and sieves, contaminated water were some of the basic determinants of milk quality. Furthermore, the milk was also subjected to more contamination as it was transported long distances to the processing plant under high ambient temperature and without cold chain facility and using materials which were not airtight.

The high level of counts and isolate numbers and types found in the milk represent a poor keeping quality and public health risk to the consumer and this suggests the need for improved hygiene practice at all levels in the dairy.

Keywords: Milk, TAPC, Coliform counts, MPN, Raw, Pasteurized, Critical points, CMT.

1. INTRODUCTION

Milk is the lacteal secretion of the mammary glands of a mammal. As it is well known, milk is the first natural food of all young mammals during the period immediately after birth (Teka, 1997). Man has consumed milk and milk products even before the dawn of civilization. Because of its high nutritive value, milk is considered as one of the most important diet items of many people (Mehari, 1988). Nutritionally, milk has been defined as "the most nearly perfect food". It provides more essential nutrients in significant amounts than any other single food. Milk is an outstanding source of calcium and phosphorus for bones and teeth, and contains riboflavin, vitamin B_6, A and B_1 in significant amounts. It also contains vitamin B_{12}, the antiperinicious anemia vitamin (O'Mahony, 1988).

As milk and milk products play an important role in human nutrition throughout the world. Consequently, the products must be of high hygienic quality. In less developed areas and especially in hot tropics high quality of safe product is most important but not easily accomplished (DeGraaf *et al*., 1997). This is required since milk is also a suitable substrate for microbial growth and development. The fluid or semi-fluid nature of milk and its chemical composition (containing the essential nutrients) renders it one of the ideal culture media for microbial growth and multiplication (Gudeta, 1987; Ashenafi and Beyene, 1994; Soomro *et al*., 1996, Teka, 1997). Mainly because of this reason, milk and milk products are more prone to the harbouring and proliferation of microorganisms.

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources i.e., within the udder, the exterior of the udder and the surface of the milk handling and storage equipment (Murphy, 1995; Godefay and Molla, 2000).

Microorganisms may contaminate milk at various stages of milk procurement, processing and distribution. The health of the cow and its environment, improperly cleaned and sanitized milk handling equipment, and workers who milk cows and come in contact with milk due to a number of reasons could serve as sources of microbial contamination of milk. Use of non-potable water may also cause entry of pathogens into milk. It is known that tropical conditions which have a hot, humid climate for much of the year are ideal for quick milk deterioration so

pose particular problems because the temperature is ideal for growth and multiplication of many bacteria (Gilmour, 1999; Godefay and Molla, 2000).

Although milk is known to posses several antimicrobial systems, bacterial numbers will double in less than 3 hours in unchilled milk. The rate of microbial growth will depend on initial numbers and the temperature at which milk is held after milking and thereafter (Kurwijilla *et al*., 1992).

The increase in urban populations during the present century and improvements in methods of milk preservation have led to large scale transportation of milk from the producer to the consumer areas (Linton, 1982). Raw milk collection and its transportation to the processing centers present a number of technical, economical and organizational problems in most developing countries in tropical regions. These have inevitably increased the risk of infection of many people from a common source. Lack of refrigeration facilities at the farm and household level, with high ambient temperature implies that raw milk will easily be spoiled during storage and transportation (Kurwijilla *et al*., 1995; Ombui *et al*., 1995; Gilmour, 1999; Godefay and Molla, 2000). The risk can be reduced by suitable precaution. Methods employed for improving the keeping quality of milk are often adequate to render safe for consumption (Linton, 1982).

Diseases that commonly spread from the milk to human beings are tuberculosis, brucellosis, salmonellosis, listeriosis, campylobacteriosis, yersinoses and Q-fever. Other bacterial pathogens transmitted to humans include *Streptococcus agalactae*, *staphylococcus aureus*, and *Escherchia coli* (Hahn, 1996).

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*, etc. Various other pathogenic causing diseases like cholera and typhoid may find access in the milk from various other sources, which may include water, and the persons handling the milk. Nonpathogenic microflora may come directly from the udder and may also enter in the milk from milker's hands, utensils, cow barn, water, etc. (Hahn, 1996).

In Ethiopia the average lactation milk yields per cow in indigenous cattle is estimated to be 213 Kg of milk. At present, the per capita consumption of milk estimated is to be 10 Kg, which is lower than other countries in the region (CTA, 1997). Total annual milk production is estimated to be 830,000 tons of raw milk equivalent of the total national production, 85-89% is obtained from cattle (CTA, 1997). Milk in most places in Ethiopia is consumed raw. Milk products such as yoghurt, butter and buttermilk are also produced using raw milk as a starting material. Hence there exists the possibility of consuming milk, which has been contaminated with disease causing organisms (Mehari, 1988).

The actual methods of storage on the farm and mode of transportation to the processing plant don't prevent microbial activity in the raw milk in the country. Pasteurization and post - pasteurization handling and marketing of milk has its own problems. Hence the keeping quality is also low. Due to either surviving thermoduric bacteria and/or post pasteurization contamination, the shelf life of pasteurized milk at ambient temperature or refrigeration temperature decreases through time (Mehari, 1988). Hygienic quality control of milk and milk products in Ethiopia is not usually conducted on routine basis. Apart from this, door-to-door raw milk delivery in the urban and peri-urban areas is commonly practiced with virtually no quality control at all levels (Godefay and Molla, 2000).

In countries with poor milk production and marketing practices, one can expect high initial viable bacterial counts posing a health hazard as well as spoilage of large quantities milk (Hailu, 1989). The number of bacteria may increase considerably if samples taken at the time of milking from the cows and distribution to the consumers are tested for total bacteriological counts. Information on the bacterial content of a milk sample may reflect on the state of health of the cow, the contributions under which the milk is stored and distributed, and its public health significance (Malick, 1986). The present study were carried out, with the following objectives:

- To detrmine some of the critical control points in milk handling,
- To estimate the microbial load of milk at each critical control points and farm water and
- To isolate and identify bacterial pathogens, in milk samples.

2. LITREATURE REVIEW

2.1. Bacteriological quality of raw milk

Due to its complex biochemical composition and high water activity, milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms (Ashenafi and Beyene, 1994; Soomro *et al*., 1996). Presence and multiplication of saprophytic bacteria in raw milk might change the milk composition and influence the quality of the product (Godefay and Molla, 2000). Moreover, the flavor of the raw milk may be adversely affected and heat stable bacterial enzymes may continue to act in the product, particularly during long storage and adversely affect stability and/or flavor of cream and upper heat-treated milk (Heeschen, 1994).

2.1.1. Sources and significance of bacterial contamination

The bacterial contamination in milk emanates from a number of sources including mastitis, external udder surfaces and from the milking plant (Hagstad and Hubbert, 1986; Ashenafi and Beyene, 1994; Slaughuis, 1996). Inadequate cooling of the milk, improper udder preparation methods, unclean milking equipment and the water used for cleaning purposes are considered as the main source of milk contamination (DeGraaf *et al*., 1997). In order to produce milk of good bacteriological quality, dairy farmers should be aware of the sources of contamination and importance of proper milk handling, cooling and storage.

2.1.1.1. Interior of the udder

2.1.1.1.1. Healthy udder

For many years, it was believed that milk drawn directly from the udder of a healthy cow was a sterile fluid, that is, it contained no living microorganisms. It has been demonstrated; conclusively that freshly drawn milk usually contains bacteria (Murphy, 1996; Walstra *et al*., 1999). The numbers of bacteria, which are present in freshly drawn milk, vary with individual animals, quarters of the udder, environment of the animal (cleanliness of quarters), health of the animal, and other factors. It is found that the first milk withdrawn from the udder (foremilk) usually has a higher bacterial content than that drawn later in the milking process,

while the strippings may show a somewhat higher count than the latter (Murphy, 1996; Walstra *et al*., 1999).

Raw milk as it leaves the udder of healthy cows normally contains very low numbers of microorganisms and generally will contain less than 1000 total bacteria per ml (Murphy, 1996; Godefay and Molla, 2000). Natural flora within the udder of healthy animals is not considered to contribute significantly to the total numbers of microorganisms in the bulk milk, nor the potential increase in bacterial numbers during refrigerated storage. Natural floras of the cow generally have little influence on standard plate counts (SPC) (Hagstad and Hubbert, 1986; Murphy, 1996).

2.1.1.1.2. Infected udder

In case of mastitis counts of *Streptococci*, *Staphylococci* or coliforms will be as high as the total plate count and can be very high up to 10^7 cfu/ml. Bulk milk count may even increase to 10^5 cfu/ml under certain circumstances (Slaghuis, 1996). Table 1 shows some of the pathogenic bacteria of public health significance from infected udders of cows.

2.1.1.2. Exterior of the udder

The exterior of the udder can be an important source of contamination. But the exterior of the udder is influenced by the environment of the cows, in which cows are housed and milked (Murphy, 1996).

Table 1: Pathogenic bacteria of public health significance from infected udder of cows

Pathogenic bacteria	Remark
Mycobacterium bovis / Mycobacterium tuberclosis	Tuberculosis
Brucella abortus / Brucella melitensis	Brucellosis
Coxiella brunetii	Q-fever
Infected Udder	
Staphylcoccus aureus	Enterotoxin
Escherchia coli	Some serotypes pathogenic to man, fecal contamination
Streptococcus agalactae	Pathogenicity for man uncertain
Infected udder minor	
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)

2.1.1.2.1. Housing conditions

In temperate regions, cows are housed in winter and pastured in summer. Differences in teat contamination can be found between housing and pasturing. Both total plate and aerobic spore counts are lower when cows are at pasture. When cows are housed, bedding material and feed stuffs can be contamination sources. In both cases (housing and pasturing) feces and dung are also an important contamination sources. Contamination of bedding material can be very high due to absorption of urine and feces (Slaghuis, 1996).

2.1.1.2.2. Teat contamination

The groups of microorganisms isolated from teats are mainly Microcoocci and aerobic spore formers. The method of sampling teats can give different results but in general most bacteria found are aerobic spore formers. This can be a problem in producing milk in that the spores may survive pasteurization temperatures and spoil the milk and milk products during storage

(*Bacillus* spores) and semi-hard cheese during ripening (clostridial spores). Teat surfaces are also sources of clostridial spores in milk. Sources of these spores are feed stuff, silage and bedding. The number declines markedly when cows go out to pasture because the pasture environment is cleaner than housing conditions (Slaghuis, 1996).

2.1.1.2.3. Udder preparation

Careful cleaning of the cow prior to milking significantly reduces contamination. Clipping the flanks, escutcheon, and udder reduces contamination from hair and adhering debris (Hagstad and Hubbert, 1986). A maximum reduction of teat contamination of 90 % can be achieved with good udder preparation (washing with disinfectant and drying with paper towel) before milking. This depends on the initial level of contamination and the way of udder preparation. So with high initial contamination levels this 90 % reduction might not be reached (Murphy, 1996).

2.1.1.3. Milking and storage equipment

During milking, the major source of bacteria in milk is the milk contact surfaces of milking equipment and milk cans or bulk tanks (Ashenafi and Beyene, 1994). In practice, the contribution of milking equipment to the microflora of the milk can't be accurately obtained by bacteriological counts on the milk produced, because of the variability in numbers and types of bacteria derived from cow's udder (Hagstad and Hubbert, 1986; Murphy, 1996).

2.1.1.3.1. Cleaning and disinfections of equipment

Cleaning and disinfections of equipment after each milking is important for reduction of contamination of milk from the equipment and with rinsing, about 10 % of the number of bacteria found in milk can be reduced (Murphy, 1996). In most cases not all bacteria are removed and killed during cleaning and disinfections. Also out growth of remaining bacteria fixed in the wall of the container between two milking is supposed (Mehari, 1988). A milk can that is improperly washed, inadequately sanitized or sterilized or insufficiently dried, may contribute millions of bacteria to every milliliter of milk placed in it (Murphy, 1996).

2.1.1.3.2. Storage of raw milk

After production, milk can be stored in cans and in bulk tanks before collection. Raw milk stored in cans will be transported to the dairy plant on the same day, because storage temperatures are rather high. Spoilage of this raw milk is due to Streptococci and coliforms resulting in souring of the milk. Milk storage transport are aimed at having good quality milk available where and when needed for processing (Walstra *et al.*, 1999).

2.1.1.4. Miscellaneous sources of bacteria in raw milk

Although the air of the milking environment rarely contributes a significant number of the total microbial count of milk, extremely dusty conditions may increase the counts. Milk handling personnel (milker, butter maker, cheese maker, etc.) may contribute various organisms including pathogens especially when they are careless, uninformed, or willfully negligent, directly to milk (Ashenafi and Beyene, 1994). Polluted water may also cause entry of pathogens into milk (Gudeta, 1987; DeGraaf *et al.,* 1997). The soils, while the cows are in pasture, manure, the animal coats, tails etc. are some of the possible sources of contamination of milk (Teka, 1997). Substances such as salt, water, etc., added to various dairy products, may be a source of microorganisms in large or small numbers, and of harmless or harmful types (Murphy, 1996; Walstra et al., 1999).

2.1.2. Cooling of milk

To prevent or retard growth of bacteria in milk and to maintain its quality for domestic consumption or during transport to the processing plant, it is essential to cool the fresh milk as quickly as possible (Godefay and Molla, 2000). Although milk is known to posses several natural antimicrobial systems, bacterial numbers will double in less than 4 hours in unchilled milk. The rate of microbial growth will depend on initial numbers and the temperature at which milk is held after milking and thereafter (Kurwijilla *et al.*, 1992). In the tropical countries of Africa with high ambient temperatures, lack of refrigeration facilities at the farm and house hold level imply that raw milk will acidify very fast unless and otherwise protected (Godefay and Molla, 2000). Therefore the collection systems must be designed to move the milk to the cooling and/or processing center in shortest possible time. In addition every effort should be made to use available systems such as water cooling, air circulation or shaded areas to reduce milk temperature (DelloCastillo, 1990).

2.1.3. Bacteriological quality tests

Sanitary methods of handling milk must be strictly adhered to rigidly in order to provide safe milk for human consumption. Furthermore, since milk is a good growth medium, even a small number of non pathogens can multiply considerably if the milk is not kept refrigerated. Because the consumer has no way of knowing whether or not the milk delivered to the home or purchased in the store is contaminated, a number of standard tests are carried out periodically on milk in that area. From the results of these tests, milk is classified into grades designated as A, B, and C (Volk and Wheeler, 1980). Tests commonly employed to determine the quality of milk include dye-reduction (Methylene blue reduction and resazurine reduction), Alcohol test, Standard plate count, Coliform count, Somatic cell count, Titrable acidity, and phosphatase tests

2.1.3.1. Dye-reduction tests

These tests are less precise criterion for classifying raw milk according to its bacteriological quality. This calls for the need to periodically verify the quality of milk with more precise microbiological tests such as standard plate count (Ombui *et al.*, 1995).

2.1.3.1.1. Methylene blue reduction test

Methylene blue is a blue-colored reagent which is used to estimate the bacterial population of a given milk sample. 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 disappears. The number and species of organisms present in the milk determines the time required for the disappearance of the blue color in the milk (Teka, 1997). Normally if the number of bacterial organisms is greater, the time required to decolorize the blue color is shorter. This test is usually used for grading the quality of raw milk before pasteurization. On the basis of this test, raw milk is graded as follows (Kurwijilla *et al*., 1992):

- Very good: not decolorizing in 5 hours.
- Good: decolorized in less than 4 hours, but not less than 3 hours.
- Fair: decolorized in less than in 2 hours, but not less than 1 hour.
- Poor: decolorized in less than ½ hour.

2.1.3.1.2. Resazurine reduction test

This test is also used for grading the sanitary quality of raw milk by applying the chemical reagent resazurin. The procedure is similar to that for the methylene blue test, except that this test is quicker and the result is obtained in much less time (Teka, 1997). Resazurin 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 comparator disc reading value of 4 and above for 10 minutes resazurin test indicates good quality but while a comparator disc reading value of less than 4 at 10 minutes indicates poor quality milk (Ombui *et al*., 1995).

2.1.3.2. Alcohol test

When milk contains more than 0.21% acid, or when calcium or magnesium compound are present in greater than normal compounds, 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 (O'Mahony, 1988; Ombui *et al*., 1995).

2.1.3.3. Standard plate count (SPC)

The standard plate count of raw milk gives an indication of the total number of aerobic bacteria present in the milk at the time of pick up. Obviously, very clean milk will have lower bacterial counts than milk collected or handled under unsanitary conditions. The standard plate count is a basis for grading milk (Volk and Wheeler, 1980). 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 (Murphy, 1996). This method is used mainly to estimate the bacterial population of raw milk prior to heat treatment. It has a limited value in that it doesn't indicate the quality of microbial populations in terms of pathogens and non pathogens (Teka, 1997). The standard plate count is generally accepted as the most accurate and informative method of testing bacteriological quality of milk (Kurwijilla *et al*., 1992; Godefay and Molla 2000). It is sensitive but also labour intensive and is inaccurate for high count milks (Slaughuis, 1996). Plate count standards have been developed to ensure satisfactory production hygiene and that the product

is safe (Table 2). The plate count method has been conducted as a valuable adjunct to guide sanitarians in correcting sanitation failures and improving milk quality (IDF, 1990).

Table 2: Grade of raw milk based on SPC

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: Kurwijilla *et al.* (1992)

The USPHS grade A pasteurized milk ordinance in the USA requires that grade raw milk for pasteurization at the farm has a bacterial count not exceeding 10^5 cfu/ml. This ordinance also requires that the bacteriological count of commingled raw milk not exceeding 3 x 10^5 cfu/ml at the time of arrival at the dairy processing plant (IDF, 1990).

2.1.3.4. Coliform bacteria in raw milk

Coliforms are group of bacteria, which inhabit the intestinal tracts of human and animals. They are excreted in large number with human excreta and animal droppings. They may be found in the soil, on vegetables and in untreated water (Teka, 1997). It includes all aerobic and facultatively anaerobic, Gram-negative, non-spore forming rods able to ferment lactose with the production of acid and gas at 35^{o}C within 48 hours. Most of them belong to the genera *Escherichia, Enterobacter* and *Klebsiella* (Godefay and Molla, 2000). The presence of coliform organisms in milk indicates unsanitary conditions of production, processing or storage. Hence their presence in large number in dairy products is an indication that the products are potentially hazardous to the consumers' health (Volk and Wheeler, 1980; Godefay and Molla, 2000). Coliform organisms contaminate raw milk from unclean milker's hands, improperly cleaned and un sanitized or faulty sterilization of raw milk utensils especially churns, milking machines, improper preparation of the cows' flecks or dirt, manure, hair dropping into milk during milking, udder washed with unclean water, dirty towels and udder not dried before milking (Ombui *et al.,* 1995).

2.1.3.5. Tests for specific pathogens

Unless there is some evidence that a particular disease is being transmitted through milk, tests for specific pathogens are not run. The procedure to be followed depends on the specific organism in question (Volk and Wheeler, 1980: Carter, 1984; Quinn, 1999).

2.1.3.6. Somatic cell counts (SCC)

The somatic cell count (SCC) is internationally recognized as a parameter for assessing milk quality and udder heath (Degraaf *et al*., 1997). EU standards require that the milk should not contain more than 400,000 somatic cells/ml. Milk markets routinely rely on somatic cell counts to ensure a quality product. Somatic cell counts levels are monitored to ensure compliance with set milk quality standards. Today, most markets in developed countries pay a premium for low SCC, good quality milk. One can appreciate the reasons, for paying a bonus for quality milk when the relationship between mastitis (high SCC) and milk composition is understood. Chemical changes in milk composition due to mastitis reduce milk quality (Rice and Bodman, 1997).

2.1.3.7. Titrable acidity test

In order to determine the sourness of milk, we use titration using sodium hydroxide (NaOH) and the degree of sourness is given by Soxhilet-Henkel Degree (SH^0). Generally the sourness of normal milk is 6 to 7 SH^0. If the milk sourness is 4 to 5 SH^0, it indicates that either the milk is adulterated or there is mastitis (Kurwijilla *et al.,* 1992).

2.1.3.8. Phosphatase test

The phosphatase test is the most important public health measure for controlling the efficiency of pasteurization, hence the safety of milk. Phosphatase is an enzyme, which is normally present in raw milk. When milk is pasteurized by any of the recognized processes, the enzyme is completely inactivated. Therefore, a positive phosphatase test will indicate that the milk is not properly pasteurized. It may mean any one of the following (Teka, 1997):

- The pasteurization temperature time combination was not strictly observed or
- The pasteurization equipment was not functioning properly or
- The pasteurized milk has been contaminated by raw milk.

This is important because improperly pasteurized milk still could transmit tuberculosis, brucellosis, and Q fever (Volk and Wheeler, 1980).

2.1.3.9. Other milk quality tests

Organoleptic tests: Microorganisms cause various undesirable and detectable organoleptic and physical changes in raw milk. Generally, when actively growing types of organism capable of causing changes in flavor and physical appearance reach population levels of 5-20 millions per ml; organoleptic and physical changes are evident or imminent (Ashenafi and Beyene, 1994). The general appearance, cleanliness, colour and smell of the fresh milk should be checked at collection before it is blended with milk from other suppliers since the volume and value at risk increases down the chain (Harding, 1999).

Sedimentation test: Performed by leaving milk in flask or any container and kept for 15-30 minutes and observing if there is any sedimentation of dirt. The sediment can be examined bacteriologically for the presence of bacteria (Warner, 1975).

Clot on boiling test: Acidity decreases the stability of milk. If the concentration of hydrogen ion is more than the normal amount (O'Mahony, 1988), then casein will get precipitated on heating immediately. The clot on boiling test is used to determine whether milk is suitable for processing, as it indicates whether the milk is likely to coagulate during processing (usually pasteurization). It is performed when milk is brought to the processing plant. If the milk fails the test, it is rejected (O'Mahony**,** 1988).

Catalase test: This measures the activity of the enzyme catalase. The catalase content of milk primarily depends up on the number of cells in milk. Hence the increased activity of this enzyme indicates mastitis (Cheesbrough, 1984).

Specific gravity: To test adulteration, specific gravity is measured and calculated. The specific gravity of milk will be measured using lactometer. The specific gravity of normal unadulterated cow's milk is between 1.026 and 1.032 at 20 ^{0}C (Ombui *et al*., 1995).

Freezing test: The normal freezing point of milk is between -0.50 and -0.61^{0}C. The soluble constituents, lactose and ash determine the freezing point of milk and are responsible for its being lower than that of water. This fact makes it possible to determine whether or not milk

has been watered. It had been shown that with addition of 1% of water to milk, the freezing point is raised approximately by 0.0055^0C (Hansen, 1994).

2.1.4. Public health aspects

2.1.4.1. Public health significance of raw milk and milk products

Milk, either raw or processed, is a well known vehicle of a number of human pathogens. Milk and milk products have, therefore, pose a health risk to consumers if it is contaminated by any pathogens and subjected to temperature abuse where these organisms can multiply to high counts and may produce toxins (Radostits *et al*., 1994). In countries where foodborne illness are investigated and documented, the relative importance of pathogens like *Staphyloccocus aureus*, *E. coli*, *Salmonella* species and *Listeria* species is well known (Godefay and Molla, 2000). The following are the most important disease causing organisms that can be found in milk.

*Mycobacterium bovis***:** causes tuberculosis in both human and domestic animals. The disease is important from the point of public health as well as its detrimental effects on animal production. When raw milk is consumed, it acts as a vector by which *M. bovis* is transmitted to man (Sinha, 1994a). Human tuberculosis due to *M. bovis* is rare in countries that have adopted pasteurization and extensive veterinary control measures but even in those countries the disease is not extinct (Sinha, 1994a). This should not lead to any complacency about the potentially serious health hazard of *M. bovis* infection through milk. The situation in developing countries may be much more demanding because of the higher incidence of *M. bovis* in dairy animals resulting in dissemination of the organism through raw milk (Sinha, 1994a).

Mycobacterium tuberculosis: causes tuberculosis in humans, the organisms get access to milk primarily from infected producing animal or infected humans or animals at post secretary stage (Sinha, 1994b). Human tubercle bacilli apparently cause transient infections in cattle. In such cases, cattle may excrete the bacilli in their milk from apparently normal udders. There have been few reports of symptomless cows excreting virulent *M. tuberculosis* in their milk following inoculation and natural infection (Sinha, 1994b). Raw milk consumption is thus the most important channel for the transfer of tubercle bacilli.

Salmonella species: Most foodborne salmonellosis outbreaks have been implicated to food containing eggs or poultry products. Nevertheless, there have been several outbreaks of salmonellas for which milk or milk products were responsible (Vlaemynck, 1994). Natural infections of the udder may occur very rarely and therefore don't play any role in human infections. There are a lot of case reports about epidemics by consumption of raw milk, however, mostly from the seventies and eighties. Contamination of raw milk mostly is due to infected persons and to environment.

Brucella species*:* From the view point of human health, the disease is important because the organism can cause undulant fever or Malta fever or Mediterranean fever in man. The possibility of infection occurring by drinking of infected milk necessitates the pasteurization of milk. Officially approved method of commercial pasteurization renders naturally *Brucella* contaminated raw milk safe for consumption (Radostits *et al*., 1994; Garin-Bastuji *et al*., 1994).

Escherchia coli: This is frequently contaminating organism and is reliable indicator of fecal pollution generally in sanitary conditions of water, food and milk and other dairying products (Soomro *et al.,* 2002). Recovery of *E.coli* from food is an indicative of possible presence of entero-pathogenic and/or toxigenic microorganisms, which could constitute a public health hazard. *Escherchia coli* is frequently occurring organisms in milk whenever the methods of production, transportation, handling and sale of milk are unhygienic. The milk sold in raw forms and because of possibilities of contamination with *E. coli* poses a great hazard to public health *(*Rea and Fleming*,* 1994).

Listeria monocytogenes: Dairy products have been cited frequently as causes of food poisoning and the industry is now required to go to the extensive expense to ensure the dairy products are free from *Listeria monocytogenes*. *Listeria monocytogenes* is widespread in nature and frequently gains access to the farm milk supply. This can either be from infected animals or more frequently from the environment in which the milk is produced. Direct contamination from milking equipment is also a source of *Listeria monocytogenes* in raw milk (Prentice, 1994). *Listeria monocytogenes* mastitis is rare. However, it poses a serious public health risk, particularly where dairy industry use raw milk. The risk is due to both the persistence of infection and the level of milk contamination. Consequently in order to prevent contamination early detection of cows with *Listeria* mastitis is required (Sana *et al*., 1996). Good hygienic practice during milking and proper use of normal cleaning routines are

adequate to limit the number of *Listeria monocytogenes* in milk as it has no special resistance to the routinely used cleaning material. The organism can grow in milk at refrigeration temperature but it has been now demonstrated that all *Listeria* species are destroyed by pasteurization. Problems with *Listeria* species in milk therefore are limited to post pasteurization contamination (Prentice, 1994).

Coxiella burenetii: It causes Q-fever in human after the consumption of raw milk. Although *Coxiella* is more resistant to heat treatment than *M. tuberculosis*, it is inactivated by usual pasteurization procedure. In humans, the transmission path way is 90% to 95% aerogenic by dust and per orally by contaminated food, which is less important, if it is mainly caused by contaminated milk and milk products. The number of *Coxiella* necessary for infection is rarely found in this kind of food. Nevertheless, milk is to be seen as a relatively high risk within food, especially in warm countries with a high incidence of Q-fever in domestic animal (Hahn and Koch 1996).

Staphylococcus aureus: It is the leading cause of foodborne illness throughout the world. Milk and milk products can became contaminated unless good hygiene (including mastitis) control occurs on farms, the milk is adequately pasteurized, and precautions are taken to prevent contamination and subsequent growth of *Staphylococci* during the manufacturing process and the finished product. The pathogenicity of *Staphylococcus aureus* has been recognized for many years and it may cause mastitis or skin disease in milk producing animals or lead to foodborne intoxication in milk and milk products (Asperger, 1994). Human carriers can also contaminate milk. Five serologically distinct enterotoxins (A, B, C, D, and E) are recognized, with enterotoxin A most frequently involved in food poisoning outbreaks. The minimal intoxication dose is 100 nanogram and sometimes less (Asperger, 1994).

Campylobacter jejuni: Among the organisms known to be the cause of enteritis in man and animals, *Campylobacter jejuni* was not recognized until the beginning of the 1980's. In the mean time this species has been established as one of the most common food borne bacterial pathogens comparable to *Salmonella* (Hahn, 1996). No symptoms of mastitis could be detected, but the consumption of raw milk of infected cows caused enteritis in some persons. The relation between the very rare findings of naturally infected udders and the incidence of infections in humans by raw milk indicates a post secretary contamination (Hahn, 1994a). Regarding the pathogenicity for humans it is to be mentioned that *Campylobacter jejuni* has a very low infection dose of about 500 viable organisms. In spite of the high contamination rate

in slaughter animals especially in poultry (up to 100%) and the possibility of human infection by contaminated meat, the transmission of *Campylobacter jejuni* by the consumption of raw milk is obviously the most important path way in the epidemiology of human campylobacteriosis (Hahn, 1994a).

Yersina enterocolitica: Among the *Yersina* species in food hygiene, only *Yersina enterocolitica* is of predominant importance. Obviously, milk and milk products as a source or a vehicle for human Yersiniois are not so important as for example with *Campylobacter* infections. Because *Yersina enterocolitica* is surely inactivated by pasteurization, only raw milk and milk products or recontaminated ones may be dangerous (Hahn, 1994b).

Streptococcus agalactiae: Milkborne *Streptococcus agalactae* infections are usually acquired by drinking milk from an infected cow. The organisms enter through the teats and teat canal and cause inflammation of the teats and udder of the cow. The infected cow secretes with the milk viable organisms, which eventually reach the consumers (Teka, 1997; Becker, 1994.).

Streptococcus pyogenes: This pathogen is directly responsible for a variety of inflammatory and supurative conditions such as sore throat, scarlet fever, cellulitis, erysipelas, impetigo, puerperal sepsis, otitis media, septicemia and wound infections; it is indirectly associated with rheumatic fever, glomerulonephritis and erythema nodosum. It is also found in the throat or nasal cavity in a proportion of apparently healthy carrier people (Collee *et al*., 1989).

Corynebacterium ulcerans: This organism is primarily a pathogen of animals but may occasionally infect human beings from an animal host. It can cause mastitis in cattle and has been found in milk responsible for sporadic cases or small outbreaks of ulcerative or exudative throat infection in human beings. Most of these human infections are mild but in a few patients suffers a serious illness resembling diphtheria with membrane formation and even cardiac and neurological complications (Collee *et al*., 1989).

Corynebacterium haemolyticum: It is an occasional human pathogen causing cases of pharyngitis, sometimes associated with cervical adenitis and often with a scarlatiniform rash. The disease is mild and resolves without treatment (Collee *et al*., 1989).

2.1.4.2. Public health standards for milk

The public health standard for milk ordinance provides chemical, bacterial and temperature standards as well as sanitation requirements for production and processing of grade "A" raw and pasteurized milk and milk products. Some processors may often monitory incentives for producers to meet more stringent standards to improve milk quality (Hagstad and Hubbert, 1986).

The microorganisms present can originate from interior of the udder, its exterior and/ or milking equipment. High initial microbial count in milk of $>10^5$ cfu/ml in evidence of serious faults in milk production hygiene, whereas production of milk having counts consistently below 10^5 cfu/ml reflects good hygiene practices (Ombui *et al*., 1995). A standard plate counts of $1x10^5$ cfu/ml has been widely adopted for good quality raw milk intended for treatment before liquid consumption. However, some other countries have adopted different standards suited to local conditions. For example, the standard plate count for America is no more than $3x10^5$ cfu/ml, while the standard for Kenya is no more than $2x10^6$ cfu/ml (Ombui *et al.,* 1995). In Sweden the accepted limit for the total number of bacteria and somatic cell count in raw milk is $1x10^5$cfu/ml and $4.99x10^3$ somatic cells/ml respectively (Maret, 1994). The standard plate count for pasteurized milk should be less than 30,000 cfu/ml (Saskatchewan, 1997).

Coliforms can rapidly build up in moist, milky residues in milking equipment, which then becomes the major source of contamination of milk produced. Coliform counts regularly in excess of 150 cfu/ml are considered generally as evidence of unsatisfactory production hygiene. However, relatively low coliform counts in milk don't necessarily indicate effectively cleaned and disinfected equipment (Ombui *et al*., 1995).

2.1.5. Milk and its keeping quality

The keeping quality of milk is influenced by the milk's microbial content and the conditions that enhance or inhibit growth of the milk's microbial flora. Shelf life of pasteurized milk is influenced by somatic cell concentration in raw milk and is indicative of abnormalities in the cow that is shorter shelf life and adverse milk flavors are the common results of elevated SCC score (Rice and Bodman, 1997). Product temperature is a major factor influencing shelf life. Product temperature must be between 4 to 5^{o}C for maximum shelf life. As a general rule, for

every 2.8^{o}C rise in temperature, shelf life is reduced by about 50% (Mehari, 1988). Compared to total aerobic plate counts of milk from farms which employed only cold water rinse (8.9 to 11.5×10^{6} cfu/ml), the use of an ordinary detergent and tap water did improve the bacteriological quality of milk only slightly (6.4 ×10^{6} cfu/ml) and also improve the keeping quality (Kurwijilla *et al*., 1992). Changes in on farm procedures of milk collection, longer refrigerated storage of raw milk at the farm, plus additional storage periods in bulk silos prior to processing may contribute to decreased shelf life of pasteurized milk (Mehari, 1988). Taste defects of pasteurized milks occur when total aerobic bacterial counts reach 10^{6} -10^{7} cfu/ml. Maintaining the quality of raw milk to extend the shelf life of milk and milk products is of greater importance. Milk processors wish to extend the shelf life of milk and milk products through the use of higher processing temperatures and also prevent recontamination by bacteria after pasteurization; a reduction in storage temperature increases the keeping quality of milk (Mehari, 1988).

In Ethiopia, smallholder milk processing is based on sour milk mainly due to high ambient temperatures, consumer's preference and increasing keeping quality of sour milk (Ashenafi, 1990). According to Mehari (1988) raw and pasteurized milk could be kept for 2 and 7.5 days at refrigeration temperature respectively whereas at room temperature it is only 0.9 and 4.3 days respectively.

2.1.6. Milk spoilage organisms

Bacteria that cause food to spoil are classified in three groups on the basis of temperatures for optimal replications: psychrophilic, mesophilic and thermophilic. Psychrophilic bacteria are primary spoilage bacteria because of their ability to multiply at low temperatures (Hagstad and Hubbert, 1986).

Psychrophilic organisms can grow at refrigeration temperatures. These are of interest to those storing milk at low temperatures. These bacteria grow slowly and mostly feed by breaking down protein (proteolysis) and fats (lipolysis). They can develop in raw milk during cold storage, and high numbers (in excess of 3 million cfu/ml) can produce enough enzymes to cause flavour defects. The psychrotrophic bacteria are killed by pasteurization, however, the enzymes they produce can survive. Since most strains of psychrotrophic bacteria and in particular Pseudomonas, produce lipases and proteases that critically affect the sensory and physical quality of raw milk. Although most strains produce lipases or proteases, some strains

have been shown to produce both enzymes (Harding, 1999). They are ubiquitous in nature and contaminants of milk. Poorly cleaned and sanitized dairy farm and processing plant equipment probably constitute major foci for contamination of milk with psychrophiles (Mehari, 1988). Bacteria such as *Pseudomonas putrifaciense*, with optimal replication between –1 and 20^0C and capable of relatively rapid growth at refrigeration temperatures between 2 and 10^0C are examples of psychrophiles (Hagstad and Hubbert, 1986). Other Gram-negative bacteria most commonly found to contain psychrophilic species are *Achromobacter*, *Alcaligenes, Flavobacterium, Escherichia* and *Enterobacter* spp. Gram-negative facultative anaerobic rods of the family Entrobacteriaceae collectively referred to, as coliform bacteria have been also the most frequently isolated from refrigerated raw milk (Hagstad and Hubbert, 1986). Considerable attention has been given to the incidence of heat resistance spore forming bacteria that can grow at 7^oC or less in pasteurized milk. The spores of genus *Bacillus* have been found in nearly all milk supplies and consequently, they are major problems in heat treatment of milk. These bacteria have been isolated from raw milk and pasteurized milk products. The role of *B. cereus* in outbreaks of foodborne illness is well documented. A large number of viable cells of *B. cereus* are required to cause illness. Numbers in excess of 10^5-10^6 cfu/gm have been accounted in food suspected as causing illness. Multiplication of *B. cereus* in dairy products is not only of concern of public health hazard but also a cause of economic losses through spoilage of contaminated products (Kurwijilla *et al*., 1992). Most psychrophiles produce extracellular enzymes in large quantities and some of these are mainly lipases and proteases, which affect the storage stability of milk. Many investigators have reported heat stable proteases of microbial origin. Ultra high temperature treatment of milk creates an environment suitable for protease activity. The protease attack proteins and are responsible for the development of bitter flavor or gelatin in milk. The rancid flavor produced in milk and in milk products through the action of microbial lipases is highly objectionable to man. The rancidity is mainly the liberation of butyric acid from triglycerides by the lipases (Walstra *et al*., 1999).

Mesophilic bacteria are the groups, which grow best at normal temperature ranging from about 20 to 40 ^{o}C. They are typified by the lactobacilli that can attack the milk sugar lactose and convert it to lactic acid. Their uncontrolled growth gives rise to the souring and even curdling of milk stored at ambient temperatures (Harding, 1999)

Thermophilic organisms although, they are less harmful to health, they are responsible for changes in organoleptic properties of milk products (Hagstad and Hubbert, 1986). They can endure heat and survive pasteurization (Harding, 1999).

By far the largest groups, and those with which we are concerned are called mesophiles because their optimum growth temperature falls between 20^{o}Cand 40^{o}C, the range between normal room temperature and just slightly above normal body temperature. This group mostly contains pathogenic microorganisms (Hagstad and Hubert, 1986).

2.1.7. Milk processing: inhibition and control of microorganisms

Milk processing is a method of making milk safe for human consumption. Milk produced under sanitary conditions till contains many bacteria. Therefore, it must be treated properly before consumption. The most common methods of treating raw milk are by applying heat. There are three methods of heat treatment for milk:

Boiling: This is the easiest and most practicable method of making milk safe in every home. As soon as raw milk is produced or delivered, it should be boiled. Boiling involves raising the temperature to the boiling point and maintaining at this temperature for a few minutes. Then the milk should be cooled immediately. If it has to be stored, the temperature should be maintained below 10^{o}C. Since this may be impracticable at home, preferably the milk must be consumed as soon as possible after cooling and not stored for an extended period of time after it has been boiled and cooled (Teka, 1997; Linton, 1982).

Drying: All the water component of the milk is removed by evaporation. It is brought about by heating milk at a high temperature until only solid components remain in the form of powder (Teka, 1997).

Pasteurization: is defined by the international dairy federation (IDF) as “a process applied to a milk product with the object of minimizing possible health hazards arising from pathogenic microorganisms associated with milk, by heat treatment which is consistent with chemical, physical and organoleptic change to the product” (Harding, 1999). Application of heat is the most common methods of destroying micro- organisms in food. This is achieved through pasteurization.

The objective of pasteurization is to ensure that all pathogenic and spoilage microorganisms, commonly found in milk are completely destroyed, to safeguard the food value of milk, to ensure that other non pathogenic bacteria and certain undesirable enzymes, which may cause spoilage, are inactivated or reduced to optimal levels (Teka, 1997). This requires heating the milk to a specific temperature, holding it therefore a specified period and subsequent cooling of the product rapidly below 10^0C. High temperature short time (HTST) pasteurization consists of heating the milk at 71^0C for 15 seconds (Linton, 1982; Mehari, 1988) while the low temperature long time (LTLT) of pasteurization requires treatment at 63^0C for 30 minutes (Teka, 1997). In the ultra high temperature (UHT) method the milk is heated to at least 88^0C and held at this temperature for atleast one second then immediately cooled to atleast below 10^0C (Teka, 1997).

2.1.8. Milk quality improvement

A new possibility to improve a high bacteriological quality of raw milk at ambient temperature has been tested by the activation of the lactoperoxidase system. The lactoperoxidase system, inhibits lactic *Streptococci* and Gram-negative rods especially *Pseudomonas* (Mehari, 1988).The lactoperoxidase is a natural antimicrobial system present in milk and has been established as feasible method for a temporary preservation of raw milk and represents a new approach to the old problems of finding a suitable temporary preservative for milk when adequate cooling is not available. This an alternative method for refrigeration as a way of preserving raw milk would be beneficial in many countries can not always provided, either for economic or practical reasons. Although the long term goal in these countries is to establish complete cooling facilities throughout the milk handling system, it must be realized that this will take a considerable time to accomplish. Until, then there is an urgent need for an alternative method of preserving raw milk, primarily during collection and transportation to processing centers (IDF, 1988). It occurs naturally in bovine milk in concentration of 30 mg/liter (Zaruhg and Gnan, 1999). Lactoperoxidase in milk is activated using sodium thiocyanate as a source of thiocyanate and sodium per carbonate as a source of hydrogen peroxide (Tolemariam and Beyene, 2000), yielding various intermediate oxidizing products that exhibit antimicrobial activity. Manifestation of antimicrobial activity includes inhibition of growth, oxygen uptake, lactic acid production, dropping pH and certain bacterial enzymes (Zaruhg and Gnan, 1999).

2.1.9. Importance of hygienic quality milk

Milk is an ideal balanced food for human beings. It is not surprising therefore that it also provides an ideal medium for growth of bacteria. Bacteria finding accidental access to milk may give rise to consumer's health problems or product faults. Bacteria produce enzymes, which attack fat, protein or lactose and some of these enzymes even survive in milk after the bacteria have been killed by heat treatment, hence affecting the quality of pasteurized milk, can all be minimized by starting the manufacturing process with raw milk of good hygienic quality. The hygienic quality of milk at the point of production is also of importance from both public health and consumer perception points of view, making important for milk to be produced with a low bacterial count and the count, by adequate temperature control, is to be kept low until the point of processing (Harding, 1999).

3. MATERIALS AND METHODS

3.1. Study areas

The study was conducted in Debre Zeit with a human population of about 95,000 people. The town is located about 45 kilometers south east of Addis Ababa. The altitude is about 1850 above sea level. It is an important small town where most governmental institutions, national and international research centers are located. The soil and climate are similar to those in many highland areas in Ethiopia. The main rainy season extends from June to September with an average rainfall of 800 mm (of which 84% of rain is expected). There is also a short rainy season from March to May. The annual average temperature ranges from 12.3^{o}C to 27.7^{o}C with an over all average of 18.7^{o}C. Highest temperatures are reached in May (CSA, 2001). Pasteurized milk was taken from Mama Milk Processing Plant located at Sebeta, which is 62 Kms away from Debre Zeit in north-west direction and 22 Kms away from Addis Ababa in west direction.

3.2. Study population

In Debre Zeit there is one dairy cooperative entitled by Adaa-Liben District Dairy and Dairy Product Producer and Marketing Co-operative Society (Liability limited). This cooperative in which the study was investigated has 614 members. Each dairy cow owner has one or more lactating Holstein-Friesian cross-bred cows. The estimated average milk yield is 7.5 liters/day/cow. The number of lactating cows present at the farms ranged from 1 to 5, with an average of 3 animals per farm. All animals were identified by name. The dairy owners after they milked their cows they delivered the milk to their nearest milk collection centers of their association. The milk collection centers after collecting the milk they sold it few amounts to the local consumers and large amount delivered to Mama Milk Processing Plant.

3.3. Study methodology

3.3.1. Study type

A longitudinal study was conducted from September 2003 to March 2004. Sampling was carried out repeatedly from each critical point and samples of raw milk were pooled at the

farm on cow basis and at the milk collection centers and upon arrival at processing plant on farm basis. Pooled raw milk samples was taken from the udder of each farm and its milking bucket 3 times, from each milk collection centers 3 times and upon arrival at processing plant 12 times, farm water samples from each farm 3 times and pasteurized milk samples 8 times from a processing plant.

3.3.2. Sampling procedures

The list of all dairy members was taken from Adaa-Liben District Dairy and Dairy Product Producer and Marketing Co-operative Society and then using simple random sampling technique the twenty-seven study dairy farms were identified. The selected dairy farms four milk delivering centers were incorporated in the study. Mama Milk Processing Plant was chosen since this cooperative delivered milk to this plant only. Dairy cows in one farm dried up in the second round of sampling, then the farm number were reduced from 27 to 26. Cows of two farms again dried up in the third course of study and hence the study conducted with 24 farms. The study involved 178 raw milk of which 77 pooled udder milk and 77 pooled bucket milk from the selected dairy farms and 12 pooled farms milk from the selected collection centers and 12 pooled farms milk upon arrival at Mama milk processing plant, 100 bags of pasteurized milk and 77 farm water samples were taken. The total aerobic plate and coliform counts from milk, most probable number (MPN) of coliform counts from water and isolation and identification of bacterial pathogens from milk were determined following aseptic sampling techniques.

3.3.3. Data collection

3.3.3.1. Questionnaire survey

In the study farms, basic farm data and data on milking technique, udder preparation, milk handling hygiene and storage were collected through a structured questionnaire (Annex. 1).

3.3.3.2. Collection of raw milk sample at critical control points

Raw milk samples were collected from critical control points that are considered to be associated with the hazard, when a measurement can be conducted and when control measures can be taken inorder to reduce the hazard to an acceptable level. The presence of

bacteriological agents was assessed and total aerobic plate count (TAPC) and coliform count performed on pooled milk samples collected at the following critical control points:

1. Directly from the cows' udder at farm level,
2. From the milking bucket at farm level,
3. From storage containers at milk collection centers and
4. From transportation container upon arrival at the processing plant.

Pooled raw milk samples were collected from each critical point aseptically every two months for 6 months of study period in the afternoon time. Accordingly 178 raw milk samples were collected separately and aseptically from which 77 samples were directly from the udder, 77 samples were from milking bucket, 12 were from storage containers at collection centers and 12 were from transportation containers upon arrival at milk processing plant.

Table 3: Sampling of raw milk at each critical control points at various times from milk collection centers.

Time of sampling	Cc	N	Number of samples at each critical control points				
			A	B	C	D	Total
Sept.-Nov.	1	7	7	7	1	1	16
	2	6	6	6	1	1	14
	3	7	7	7	1	1	16
	4	7	7	7	1	1	16
Dec.-Jan.	1	7	7	7	1	1	16
	2	6	6	6	1	1	14
	3	7	7	7	1	1	16
	4	6	6	6	1	1	14*
Feb.-Mar.	1	6	6	6	1	1	14*
	2	5	5	5	1	1	12*
	3	7	7	7	1	1	16
	4	6	6	6	1	1	14*
Total	4	-	77	77	12	12	178

Cc= collection centers, N=number of farms; A=samples from pooled udder milk in a farm, B= pooled milk samples from milking bucket in a farm, C=pooled milk samples from storage containers at collection centers, D=pooled milk samples from transportation container upon arrival at processing plant, *the numbers of farms was reduced to 26 and 24 in the second and third time of sampling respectively.

During sampling of raw milk from the udder, the surface of the teat end was cleaned by wiping it with clean cotton dipped in 70 % alcohol. Before sampling from milking bucket, storage container and transportation containers upon arrival at processing plant, the milk was thoroughly mixed after which 25 ml of milk is transferred into sterile sampling bottles. At all

levels of sampling, the sampling bottles were capped, labeled with a permanent marker and stored in an ice packed cool box and transported to the Faculty of Veterinary Medicine, Debre Zeit. The acidity, density and temperature of milk was determined after each milk sample was collected using alcohol test, lactometer and thermometer, respectively and the time elapsed between measurements was also be recorded. While sampling care was also taken to prevent direct contamination of milk samples with bacteria and their subsequent growth during sampling, storage and transportation.

3.3.3.3. Pasteurized milk

A total of one hundred fresh pasteurized milk samples were bought from Mama Milk Processing Plant. Sampling was done 8 times during the study period.

3.3.3.4. Collection of water samples

Water samples were collected from water sources of all the 27 farms three times at two months interval for 6 months in an attempt to evaluate the hygienic quality of farm water used for cleaning of milk equipments. Water samples were collected according to WHO (1984) standards from the pipelines of the dairy owners which were used. The water from the tap was allowed to run for 2-5 minutes to remove any contaminating organisms by removing the stagnant water from the supply line. Then the tap was turned off and the outer side dried with clean gauze. The tap was sterilized by soaking a piece of cotton wool in alcohol, igniting it and holding it with a pair of forceps close to the nozzle. Allowing water to run for a short time, the tap was cooled and finally clean, sterile and labeled bottles were filled with 200 ml from a gentle stream of water within half of the stopper to permit mixing of the contents. The stopper was then carefully replaced to avoid contaminants. These samples were transported to the laboratory in ice packed cool box to be processed according to WHO (1984) standards.

3.3.3. 5. Screening tests

3.3.3.5.1. Specific gravity test

The specific gravity of milk samples from individual dairy owner's cans were taken while supplying milk to the milk collection centers. Measuring of specific gravity performed by filling the milk sufficiently in the cylinder. Holding the lactometer by the tip, lower it gently

into the milk. Allow the lactometer to float freely until it is at rest. Read the lactometer at the top of the meniscus. Immediately, read the temperature of the milk; this should be 20 ^{o}C . If the temperature of the milk is between 17 and 24 ^{o}C, the following correction factors were used to determine the specific gravity of milk (O'Connor, 1995).

Temp ^{o}C	17	18	19	20	21	22	23	24
	↑	↑	↑	↑	↑	↑	↑	↑
Correction	-0.7	-0.5	-0.3	0	+0.3	+0.5	+0.8	+1.1

3.3.3.5.2. Alcohol test

The alcohol test of milk samples from individual dairy owner's cans were taken while supplying milk to the milk collection centers. The test was performed by transferring 2 ml of milk samples in a test tube and into which 2 ml of 68 % ethanol was added. The tubes were shaken to mix and any clot formation was noted (Ombui *et al*., 1995 and O'connor, 1995).

3.3.3.6. Bacterial counts

Decimal dilutions of milk samples were plated on plate count agar (Merck) and violet red bile agar (Oxoid) for total aerobic plate and coliform counts, respectively following the standard procedures recommended by American Public Heath Association (1992). Each plate was marked with sample number, before shaking samples and making dilutions.

3.3.3.6.1. Test procedure for total aerobic and coliform counts of milk samples

Pre-test considerations for raw and pasteurized milk:

Before removal of the milk samples from its container, the content will be mixed thoroughly and vigorously. The following dilution standards were selected so that the total number of colonies per plate fluctuates between 25 and 250 for total aerobic plate and between 10 and 100 for coliform counts.

Total aerobic plate counts (TAPC): for raw milk from the udder: 10^{-1} -10^{-4}, milking bucket: 10^{-2} -10^{6}, storage containers: 10^{-3}-10^{-8} and upon arrival at processing plant: 10^{-4} 10^{-10} and pasteurized milk: 10^{0} -10^{-4} dilutions were used.

Coliform Counts: for raw milk from the udder: 10^{0}-10^{-3}, milking bucket: 10^{0}-10^{-4}, storage containers: 10^{-2} -10^{-7} and upon arrival at processing plant: 10^{2} -10^{7} and pasteurized milk: 10^{0} -10^{-2} dilutions were used.

While transferring raw and pasteurized milk samples from one test tube to another, sterile pipettes were used. Separate sterile pipettes were used for the different dilutions. Four, six, eight ten and five sterile dilution tubes with 9 ml of sterile saline water in them were used for TAPC counts of raw milk samples from the udder, milking bucket, storage container, upon arrival at processing plant and pasteurized milk samples respectively. On the other hand three, four, seven, eight and three sterile dilution tubes with 9 ml of sterile saline water in them were used for coliform counts of raw milk samples from the udder, milking bucket, storage container, upon arrival at processing plant and pasteurized milk samples respectively.

The test procedure for milk samples:

1. The dilution saline water was labeled with sample number and dilution number
2. One ml of the milk sample was added to dilution blanks and serially diluted by taking one ml to the next dilution bottle after mixing
3. One ml was discarded from the last dilution
4. After thorough mixing, 1 ml of diluted milk was taken from each dilution starting from the highest dilution and put on sterile labeled petridishes using sterile pipettes. The covers of the petridishes were lifted just high enough to insert pipettes. Two plates were inoculated per dilutions
5. About 10-12 ml of the melted (44 ^{o}C to 46^{o}C) plate count agar and violet red bile agar for TAPC and coliform counts, respectively were poured into each plate by lifting gently the cover of the petridish just high enough to pour the medium
6. The medium was gently mixed with the test portions in the petridish by rotating and tilting
7. After the mixture was evenly spread over the bottom of the plate, it was allowed to solidify on a level surface
8. The plates were then inverted and placed in an incubator at 37^{o}C for 48 hours
9. Incubating control plates for each sterilization lot of dilution blanks and medium were used to check sterility of the dilution water and medium (APHA, 992)

3.3.3.6.2. Test procedures for MPN coliform counts of water samples

For the detection and enumeration of coliform organisms in water the multiple tubes fermentation method were used (WHO, 1984). The method consists of adding of water to sets of tubes containing lactose broth, i.e.,

- Adding of 50 ml of water sample and equal amount of lactose broth in one tube,
- Adding of 10 ml of water sample and equal amount of lactose in 5 tubes and
- Adding 1 ml of water sample and 5 ml of lactose broth in 5 tubes.
- They were incubated at 37 ^{o}C and examined at the end of 48 hours.
- The formation of gas in any of the tubes regardless of the amount, at the end of 48 hours, constitutes a positive test.
- A stastical estimate of the most probable number (MPN) of coliform organisms in a given volume of water was obtained from table of stastical probability (WHO, 1984).

3.3.3.6.3. Reading and interpretation results:

Total aerobic plate count (TAPC): After incubation at 37^{o}C for 48 hours, all colonies including those of pin point size is counted on selected plates.

Results from plates, which contained 25 to 250 colonies per plate were recorded. If plates from two conscutative decimal dilutions yield colony counts of 25 to 250, the counts for each dilution were computed by the following formula (APHA, 1992).

$$N = \frac{\sum colonies}{\left[(1xn1) + (0.1xn2)d\right]}$$

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.

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 dilution (APHA, 1992).

In case of crowded plates (more than 250 colonies); if there were fewer than 10 colonies per square centimeter, colonies in 12 squares; select representative, 6 consecutive squares horizontally across the plate and 6 conscutative squares at right angles were counted being careful not to count a square more than once. When there were more than 10 colonies per square centimeter, colonies in 4 successive representative portions were counted. In both instances, the average numbers of colonies found per square centimeter were multiplied by the area of the plate used to determine the estimated number of colonies per plate.

When computing the total aerobic plate counts (TAPC) and coliform counts; only the first two significant digits were reported to avoid creating a fictious 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 ≥ 6 and rounded down when the third digit was ≤ 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.

Coliform count for milk: After incubation of the plate for 48 hours, purplish red colonies with bile precipitations around them were counted as coliforms. Results from only those plates, which contained between 10 and 100 colonies were recorded. Interpretations were similar with that of TAPC.

MPN of coliform count from water: According to (WHO, 1984) those water samples having count range of 0-3 were considered to be potable and those counts with four and above were considered poor quality.

3.3.3.7. Isolation and identification of bacterial agents

A loopful of the milk sample was streaked onto blood agar base enriched with 7% heparinzed sheep blood and MacConkey agar. The plates were aerobically incubated at 37 ^oC and examined for bacterial growth after 48 hours. From culture positive plates, typical colonies were subjected to Gram's stain to study the staining properties and cellular morphology. Pure cultures of a single colony type from the blood agar were transferred into nutrient agar plate.

From this, a series of biochemical tests that aided final identification of various bacteria were conducted following standard methods (Quinn *et al*., 1999) (Annex.8).

Staphylococcus species were identified based on hemolysis pattern, catalase production and coagulase test, pigment production, O-F test and fermentation of manitol, maltose and trehalose (Annex. 3).

Streptococcus species were identified based on Gram's stain reaction, catalase production, hemolysis pattern, differential growth characteristics on Edward's medium, CAMP test, fermentation pattern of manitol, sorbitol, raffinose and salicine and aesculin hydrolysis (Annex.4).

Coliform organisms were identified based on Gram's stain reaction, growth characteristics on MacConkey agar, oxidase test, reaction patterns on IMViC test, H_2S production, fermentation patterns from lactose and urease and lysine production (Annex.7).

Other Gram-negative organisms were identified based on staining morphology, growth characteristics on MacConkey agar, oxidase test, urease production, indole production, and acid production from sucrose and glucose (Annex.7).

Corynebacterium species and *Actinomyces* were identified based on staining morphology, hemolysis pattern, catalase production and acid production from glucose, lactose, maltose and trehalose (Annex.5).

Bacillus species were identified based on hemolytic pattern, staining morphology, fermentation pattern of arabinose and manitol, and citrate and VP tests (Annex.6).

3.3.4. Data management and analysis

Microsoft excel was employed for raw data entry, computation of descriptive statistics and drawings. Descriptive statistics such as mean, percentage and range were used to compute some of the data. Log $_{10}$ transformation was done before the analysis of bacterial counts. ANOVA test was used to show the association between bacterial counts at different critical points, the effect of collection centers on bacterial counts and the effect of the interactions

between critical points and collection centers on bacterial counts. Intercooled Stata 7 soft was used for ANOVA tests (Stata, 2001).

The research design was split plot experimental design (Annex.2). Collection centers were considered as plots while critical points and time of collection of samples were considered as subplots and blocks, respectively.

The ANOVA model used is the following:

$Y_{ijk} = (\mu + \alpha_i + \beta_j + \gamma ij) + (\theta + \eta_{ij} + \epsilon\ ijk)$

Y_{ijk} = (fixed effects) + (random effects)

Y_{ijk}, i=collection centers, j=critical points, k=date of sampling

$\mu + \alpha_i + \beta_j + \gamma_{ij}$: μ=mean, α_i= fixed collection center effects, β_j= fixed critical moment effects, γ_{ij}= fixed interaction between Collection center and critical points.

$\theta + \eta_{ij} + \epsilon\ ijk$: θ= block effects (dates of sampling), η ij= collection center effect with in the block,

ϵ ijk=residual error

4. Results

4.1 General data

4.1.1. Description of dairy production

All the farms encountered in this study were small holder dairy farms having 1 to 5 cross-breed (Holstein-Friesian X indigenous) lactating dairy cows. Hay, straw (teff, barely and sorghum), nug cake, linseed cake, wheat bran and wheat middling were some of the feed items in the farms. The barn was cleaned daily only by removing the feces. Cleaning with water was done on average every two weeks.

4.1.2. Udder preparation and hygiene

All dairy owners used pipeline water to clean milk equipment and to wet the cows and clean them from soil and dirt. Most farms (85.2%) had not have towels for cleaning purposes of cow's udder. Those farms that had towels, they reuse it for cleaning and sanitizing of other material. This may result in recontamination of the udder. In addition to that a single towel was used common to all cows, which may result in cross contamination of the udder. No udder was properly dried. In general the udder was not prepared properly and furthermore, in some of the farms (7.4%), the milkers were inserted their fingers into the milked milk to moisten the teat whenever it got dry while milking. Milkers were not seen washing of hands between cows. The use of detergents for cleaning of milk equipment was not observed in the majority of the farms (77.8%). Hot water which is indispensable for cleaning milking equipment was not available in the 81.5% of the farms. Most of the milkers' were illiterate and they did not worry about the hygiene of milk.

4.1.3. Milking technique, milk containers and transport

All dairy cow owners milk their cows by hand and all did not cool the milk. They simply supply it to their nearest milk collection center of the association's two times in a day (morning and evening). All the farms included in this study had plastic containers for milking and transporting the milk to milk collection centers. The morning and evening milk collected at the collection centers was delivered to Mama Milk Processing Plant, without cooling and in

plastic containers, by cars. At the level of the milk collection centers, soft plastic materials which were not washed and improperly handled were used to tighten the lid covers of the milk collection containers.

4.1.4. Location of milk collection centers

Except one milk collection center all were located along roadsides which could likely exposed the milk to dust contamination created by moving vehicles.

4.2. Milk data

4.2.1. Specific gravity and alcohol tests

The specific gravity values of milk were taken from the selected farms while they delivered milk to the milk collection centers. The values were in the range of 1.025 to 1.029. The specific gravity of normal ranges betweeen1.026 to 1.032 at 20^{o}C. The majority of the samples in this study had specific gravity values generally within the acceptable range. To the contrary, (9 %) of the samples had values below 1.026, an indication of some adulteration by addition of water. However the milk collection centers were accepted 1.025 as normal parameters for specific gravity of milk. All the milk samples were negative to the alcohol test at the milk collection centers and at the same time upon arrival at Mama Milk Processing Plant.

4.2.3. CMT results

Samples examined for mastitis 15 (60 %) were negative, 4 (16 %) were trace, 5 (20 %) were weak positive and 1 (4 %) was strong CMT positive. According to Millers and Kearner (1967) trace CMT grades were considered as negative. Therefore 24% of the samples out of the total 25 farms examined were positive for CMT.

4.2.4. Bacterial counts

4.2.4.1. Milk bacterial counts

The mean ± standard error for total aerobic plate counts (expressed in log $_{10}$ cfu/ml) of raw milk sampled at four critical points are shown in table 4. There was an increasing trend of total aerobic plate counts as the milk passed through udder, milking bucket, collection centers and upon arrival at the processing plant. Results of analysis of variance indicated that there were very significant differences in total aerobic plate counts ($P < 0.001$) between the critical points and there was no significant variation between collection centers for TAPC ($P > 0.1$).

Table 4: Mean (± standard error) of total aerobic plate counts (TAPC) of pooled milk sample (log $_{10}$ cfu/ml)

Collection centre	Critical control points of sampling			
	Udder	Bucket	Storage container	Upon arrival at processing plant
1	4.53 (3.33)	5.95 (5.76)	7.68 (7.59)	8.5 (8.14)
2	4.78 (4.32)	6.62 (6.33)	7.66 (7.40)	10.13 (10.12)
3	5.63 (5.33)	6.98 (6.69)	7.73 (7.40)	9.44 (9.41)
4	5.50 (5.46)	7.71 (7.40)	8.66 (8.22)	9.72 (9.48)

The mean ± standard error coliform counts (expressed in $\log_{10}$ cfu/ml) of raw milk sampled at four critical points are shown in table 5. There was an increasing trend of coliform counts as the milk passed through udder, milking bucket, collection centers and upon arrival at the processing plant. Results of analysis of variance indicated that there were significant differences in coliform counts ($p < 0.001$) between the critical points .and there was no significant variation between collection centers for coliform counts ($P > 0.1$).

Table 5: Mean (± standard error) of coliform counts of pooled milk samples (log 10 cfu/ml)

Collection center	Point of sampling			
	Udder	Bucket	Storage container	Upon arrival at processing plant
1	3.56 (3.48)	4.49 (4.50)	5.73 (5.63)	7.04 (6.88)
2	3.94 (3.92)	5.88 (5.66)	6.68 (6.63)	7.16 (6.67)
3	3.08 (2.84)	4.56 (4.31)	5.50 (5.15)	6.58 (6.13)
4	4.68 (4.67)	5.10 (4.86)	6.03 (5.75)	7.32 (7.02)

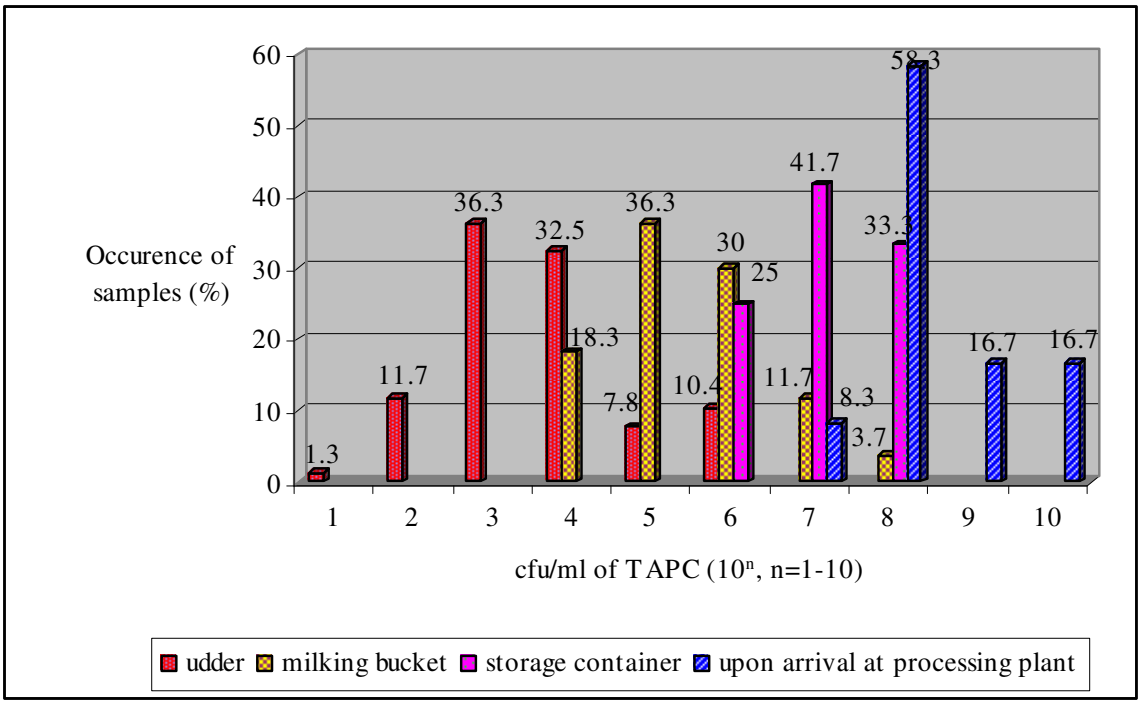
The mean ± standard error for total aerobic plate and coliform counts (log $_{10}$ cfu/ml) at three different times of sampling are presented in table 6. It is possible to see in the table that there was no significant differences in both counts at all critical points among the different times of sampling ($P > 0.1$).

Table 6: Mean (± standard error) of TAPC and coliform counts of pooled raw milk different critical points and times of sampling (log $_{10}$ cfu/ml).

Type of count	Point of sampling	Date of sampling		
		Sep-Nov	Dec-Jan.	Feb-Mar.
TAPC	Udder	4.30 (3.97)	5.59 (5.23)	5.38 (4.35)
	Bucket	7.44 (7.19)	6.87 (6.59)	7.09 (7.07)
	Storage containers	8.35 (8.26)	8.21 (7.79)	7.82 (7.78)
	Upon arrival at processing plant	10.05 (9.98)	9.69 (9.41)	8.29 (7.68)
Coliform count	Udder	3.78 (3.71)	3.56 (3.35)	4.58 (4.57)
	Bucket	5.31 (4.97)	5.58 (5.47)	4.78 (4.63)
	Storage container	6.59 (6.50)	5.56 (5.22)	5.89 (5.52)
	Upon arrival at processing plant	7.08 (6.81)	6.87 (6.60)	7.26 (6.88)

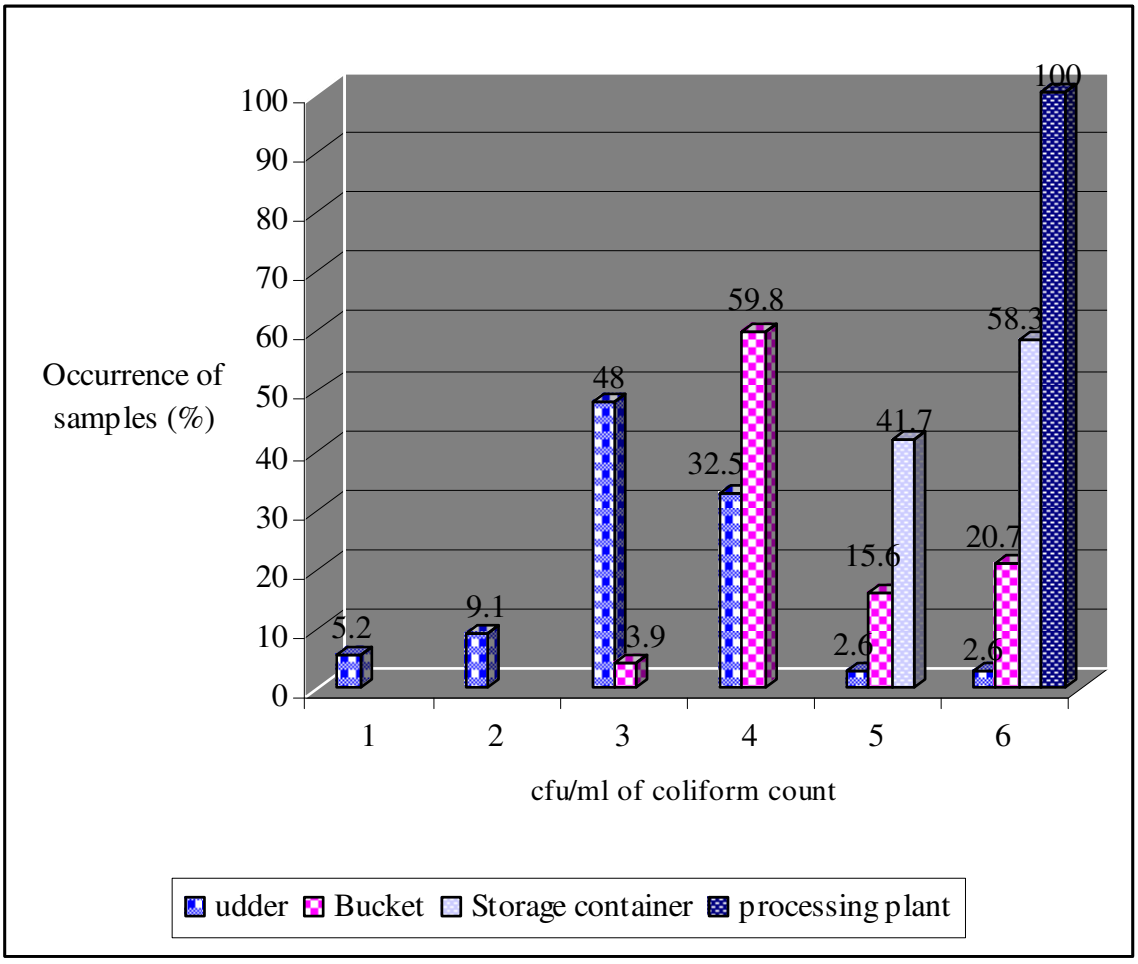
The number of milk samples falling into different categories of total aerobic plate counts are presented in figure 1. It has been found out that 10.4% of the raw milk samples from udder, 45.4% of raw milk samples from milking bucket and 100% of raw milk samples from storage containers and upon arrival at processing plant had TAPC counts > 10^5 cfu/ml which is higher than the given international standard set for minimum acceptable level of bacterial count in milk. In other words, the above indicated percentages of milk samples were considered to be below the standard set for good quality milk (Figure 1).

Figure1: Proportion of different ranges of TAPC counts in raw milk



In the case of coliform count, 37.7 % of raw milk samples from udder, 96.1% raw milk samples from bucket and 100 % of raw milk samples from storage container and upon arrival at processing plant had counts > $1.5x10^2$ cfu/ml which is higher than the given international standard set for acceptable coliform counts (Figure2).

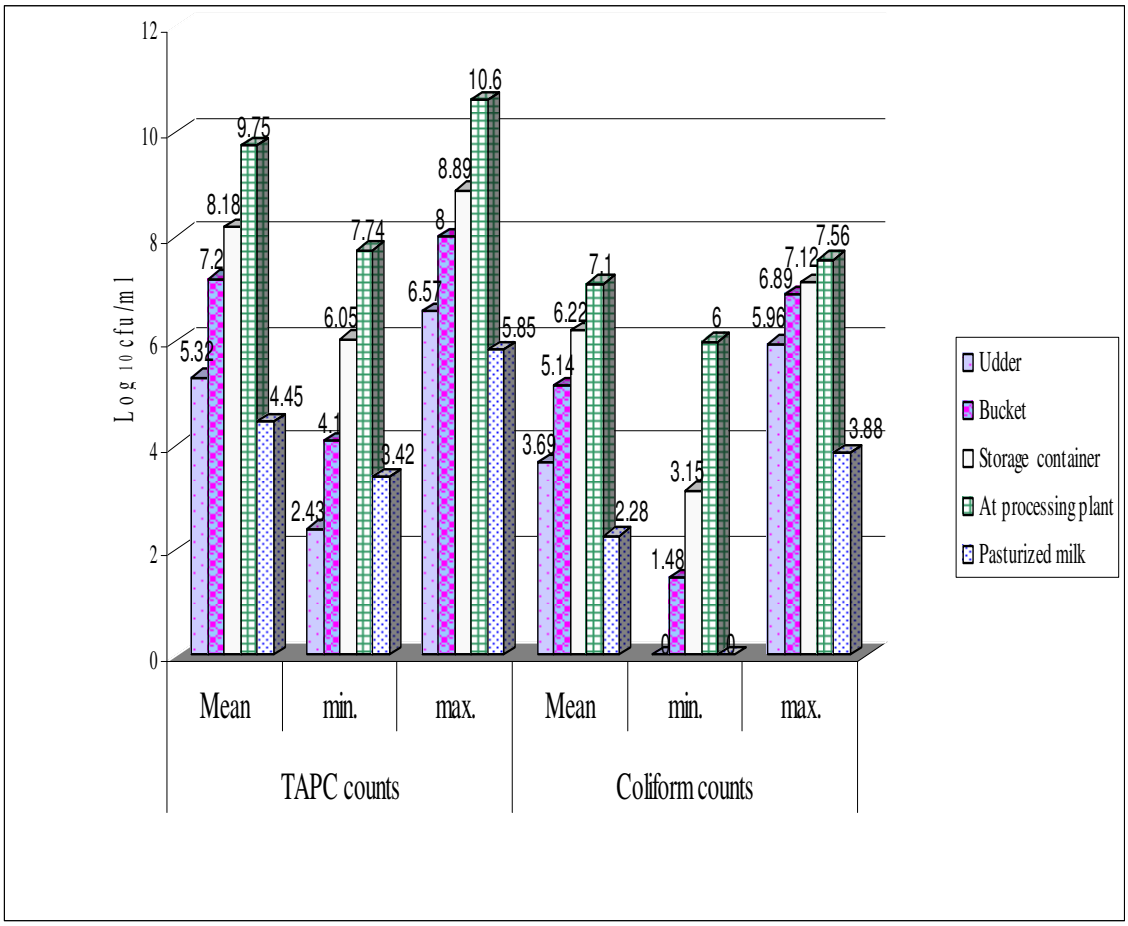
Figure 2: Proportion of different ranges of coliform counts in raw milk



Cfu/ml of coliform counts: 1=0, 2 <10, 3 = 10-150, 4 = 151-1000, 5=1001-10,000, 6 >10,000

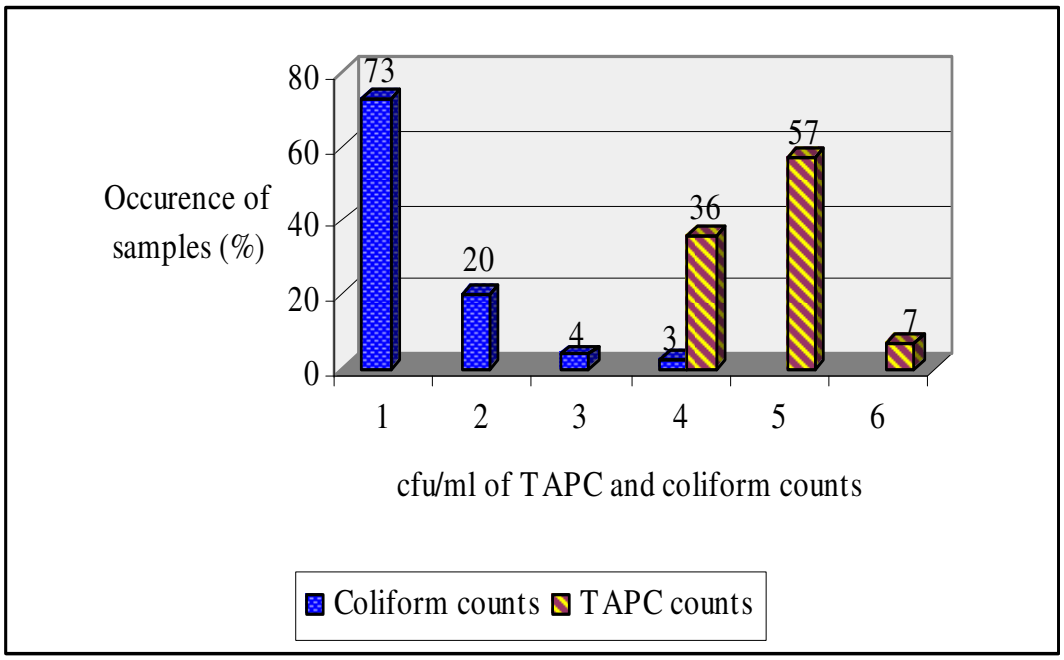
Figure 3: depicts the mean, minimum and maximum values for total aerobic plate count and coliform counts of raw and pasteurized milk. The mean values for total aerobic and coliform counts were increased accordingly from udder to upon arrival at processing plant and became lower than those of all the critical moments after pasteurization.

Figure 3: Comparison of mean, minimum and maximum TAPC and coliform counts of raw and pasteurized milk (log $_{10}$ cfu/ml).



The pasteurized milk samples falling in the different categories of total aerobic plate count and coliform count are indicated in figure 4. Pasteurized milk samples of 27% for coliform counts and 7% for TAPC had counts greater than 10 cfu/ml and greater than $3x10^4$ cfu/ml respectively, which is higher than the given international standard set for both counts. This indicates that the above mentioned percentage of pasteurized milk samples is not fulfilling the international standard for quality milk.

Figure 4: Proportion of different ranges of TAPC and coliform counts of pasteurized milk.



Cfu/ml of TAPC and coliform counts (1 $\leq 10^1$, 2 $>10^1$, 3=10^2, 4=10^3, 5 $<3 \times 10^4$, 6 $<3 \times 10^4$)

Table 7: presents the time of sampling and the temperature of milk at the different critical control points. The temperature of milk that was taken directly from udder was assumed to be the cow's body temperature and hence 38.5 ^{o}C considered as the temperature of milk from udder. Then, on average, after 23 minutes of sampling on bucket milk, the temperature became 32.4 ^{o}C to 32.8^{o}C. The temperature of milk further declined as the milk passed through collection centers (29.5 ^{o}C to 29.8^{o}C) and arrived to the processing plant (21.7 ^{o}C to 28 ^{o}C) after 53'-55' and 2h17'-2h55' of sampling respectively.

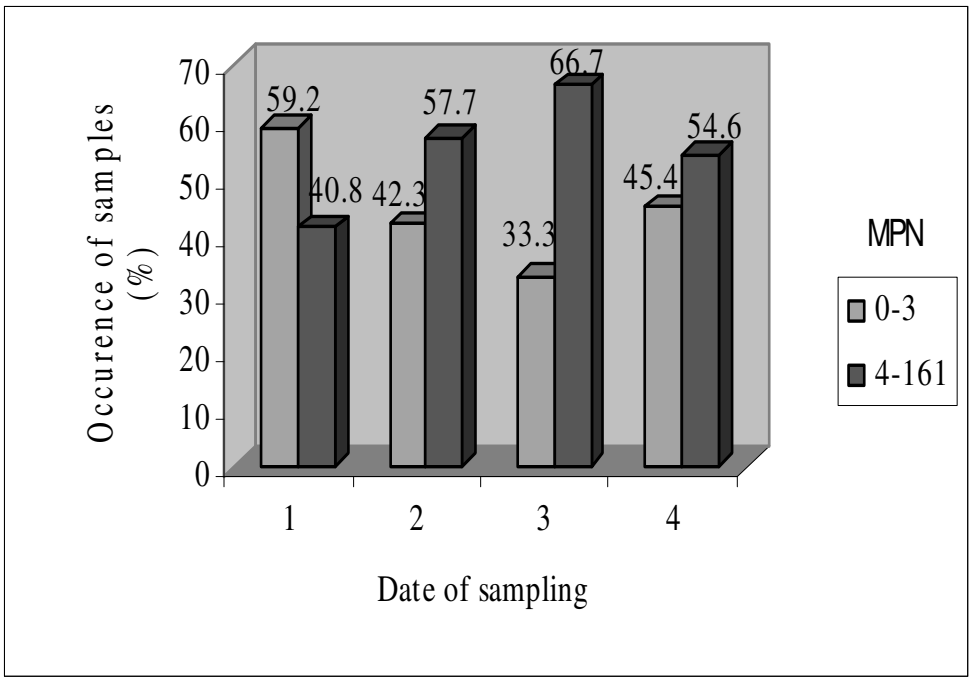
Table 7: Temperature of milk at critical points versus time of measurements.

Time of milking	Milk temperature at critical points (^{o}C)			
	Udder	Bucket	Collection center	Processing plant
Just during milking'	38.5			
23'		32.4-32.8		
53'-55'			29.5-29.8	
2h17'-2h55'				21.7-28

4.2.4.2. Water bacterial counts

During the first, second and third time of sampling 59.2%, 42.3% and 33.3%of the water samples had drinkable quality while 40.8%, 57.7% and 66.7% had not drinkable quality respectively. This indicates that as time of sampling advances the quality of water deteriorates. 54.6% of the water samples from all the three sampling times were not potable and hence could serve as a good source of contamination of milk with bacteria (Figure 5).

Figure 5: Most probable number of coliform counts of farm water



Date of sampling 1=September-November, 2=December-January, 3=February-March, 4=total samples taken from the whole studying period from September –March.

4.2.5 Bacterial isolates

4.2.5.1. Bacterial isolates of milk samples from the cows' udder

Ninety one percent (n=70) of the samples showed growth, on the inoculated media where as nine percent of the samples (n=7) did not show a growth. The major bacteria isolated out 70 positive samples were *Staphylococcus aureus*, *Staphylococcus intermidus*, *Staphylococcus hyicus*, *Staphylococcus epidermidus, Staphylococcus chromogenes, lentus, Streptococcus agalactae, Streptococcus dysgalactae, Streptococcus uberis, Streptococcus bovis, Streptococcus zooepidermicus, Enterococcus fecalis, Corynebacterium* spp, *Actinomyces* spp, *Bacillus* spp and coliform (Table 8).

4.5.2.2. Bacterial isolates of milk samples from the milking bucket

Out of the total 77 milk samples taken, none proved to be negative. In addition to those bacteria isolated from the cows udder, the following were detected, these are *Staph. saprophyticus, Staph. simulans, Micrococcus* spp, R*hodococcus equi*, *Streptococcus pyogenes, Enterob .aerogenes, Proteus mirabilis, Flavobacterium* spp, *Serratia* spp *and Past. multicoda* (Table 8).

4.2.5.3. Bacterial isolates of milk samples from the storage containers

Out of the total 12 milk samples taken, none proved to be negative. In all of the positive samples, the types of isolated bacteria were similar to bacteria isolate in the udder and bucket except the increment of isolation rates of each bacterium (Table 8).

4.2.5.4. Bacterial isolates of milk samples from upon arrival at processing plant

The type and number of bacteria isolated upon arrival at processing were similar to bacterial isolates of milk sampled from the storage containers except *Corynebacterium bovis* were isolated at an increased rate and *Proteus vulgari*s was isolated which was not present in the previous case (Table 8).

Milk appeared to be severely contaminated with environmental bacterial agents such as *E.coli, Proteus* spp*, Citrobacter* spp, *Enterobacter* spp, *Klebseilla* spp*, Pseudomonas* spp,

Flavobacterium spp, *Serratia* spp, and *Pasteurella multicoda* as it progresses from udder through milking bucket, collection centers and upon arrival at the processing plant (Table 8).

4.2.5.5. Bacterial isolates from pasteurized milk

Out of the total 100 pasteurized milk samples taken from the plant, 6% did not show a growth, the major bacteria isolated include *Staphylococcu*s *intermidus, Staphylococcus epidermidus, Staphylococcus chromogenes, Streptococcus pyogenes*, *Enterococcus faecium, Corynebacterium* spp, *Actinomyces* spp, *Bacillus* spp and *Klebseilla oxytoca* were isolated out of the 94 cultured positive samples (Table 8).

Table 8: Bacterial isolates from raw and pasteurized milk samples

Bacteria isolated	Number of isolates by source (%)				
	Raw milk				Pasteurized milk
	1	2	3	4	5
Staph. aureus	12 (15.8)	20 (26)	9 (75)	9 (75)	-
Staph. intermidus	10 (13)	13 (16.9)	7 (58.3)	7 (58.3)	10 (10)
Staph. hyicus	1 (1.3)	1 (1.3)	1 (8.3)	1 (8.3)	-
Staph. epidermidus	21(27.3)	30 (39)	11 (91.7)	11 (91.7)	41 (41)
Staph. chromogenes	7 (9.1)	18 (23.4)	8 (66.7)	8 (66.7)	19 (19)
Staph. lentus	1 (1.3)	2 (2.6)	2 (16.7)	2 (16.7)	-
Staph. saprophyticus	-	1 (1.3)	1 (8.3)	1 (8.3)	-
Staph. Simulans	-	1 (1.3)	1 (8.3	1 (8.3	-
Micrococcus spp	-	4 (5.2)	2 (16.7)	2 (16.7)	-
Rh. equi	-	4 (5.2)	3 (25)	3 (25)	-
Strep. agalactae	8 (10.4)	12 (15.9)	7 (58.3)	7 (58.3)	-
Strep. dysgalactae	2 (2.6)	4 (5.2)	4 ((33.3)	4 ((33.3)	-
Strep. uberis	14 (8.2)	24 (31.2)	12 (100)	12 (100)	-
Strep. bovis	5 (6.5)	16 (20.8)	8 (66.7)	8 (66.7)	-
Strep. pyogenes	-	3 (3.9)	4 (33.3)	4 (33.3)	5 (5)
Strep. zooepidermicus	1(1.3)	1 (1.3)	1 (8.3)	1 (8.3)	-
Enter. faecium	-	-	-	-	5 (5)
Enter. fecalis	2 (2.6)	9 (11.7)	5 (41.7)	5 (41.7)	-
Bac. spp.	2 (2.6)	10 (13)	6 (50)	6 (50)	-
Bac. stearothermophilus	3 (3.9)	3 (3.9)	2 (16.7)	2 (16.7)	-
Bac. brevis	1 (1.3)	2 (2.6)	2 (16.7)	2 (16.7)	3 (3)
Bacillus cerus	1 (1.3)	1 (1.3)	1 (8.3)	1 (8.3)	2 (2)
Bac pumilus	-	-	-	-	1 (1)
Bac. coagulans	-	-	-	-	2 (2)
C. ulcerans	5 (6.5)	13 (16.7)	8 (66.7)	8 (66.7)	9 (9)
C. bovis	7 (9.1)	11 (14.3)	6 (50)	7 (58.3)	4 (4)
C. haemolyticum	1 (1.3)	3 (3.9)	5 (41.7)	5 (41.7)	39 (39)
Act. pyogenes	2 (2.6)	4 (5.2)	3 (25)	3 (25)	8 (8)
E. coli	3 (3.9)	18 (23.4)	10 (83.3)	10 (83.3)	-
Pseudo. aeroginossa	4 (5.2)	17 (22.1)	9 (75)	9 (75)	-
Enterob. aerogenes	-	7 ((9.1)	5 (41.7)	5 (41.7)	-
Enterob. aglomerans	1 (1.3)	9 (11.7)	6 (50)	6 (50)	-
Kleb. pneumoniae	1 (1.3)	11 (14.3)	8 (66.7)	8 (66.7)	-
Kleb. oxytoca	1 (1.3)	4 (5.2)	2 (16.7)	2 (16.7)	5 (5)
Prot. mirablis	-	1 (1.3)	1 (8.3)	1 (8.3)	1 (1)
Prot. vulgaris	-	-	-	1 (8.3)	-
Citrob. freundi	1 (1.3)	7 (9.1)	6 (50)	6 (50)	-
Flavobacterium spp	-	2 (2.6)	1 (8.3)	1 (8.3)	-
Serattia spp.	-	4 (5.2)	2 (16.7)	2 (16.7)	-
Past. multicoda	-	1 (1.3)	1 (8.3)	1 (8.3)	-
Total isolates	118	291	169	171	154

1 = Udder, 2 = Bucket, 3 = Storage container, 4 = upon arrival at processing plant, 5 = packed milk after pasteurization

5. DISSCUSSION

The results of this study showed that few of the dairy cow owners used detergent and warm water for cleaning milk equipments. In contrary most of the dairy cow owners cleaned milk equipments with cold water without detergent after each usage, which may lead to insufficient cleaning and hence could serve as a major cause of milk contamination. In addition, the entire dairy cow owner's in the present study cleaned their cows' udder only with cold water and did not perform the cleaning sufficiently and properly since the time elapsed for cleaning of the udder should be until the washed water becomes clean but this was not observed in this study. It was reported by Galton *et al*. (1986) that premilking udder preparations play an important part in the contamination of milk during milking. Most of the dairy owners did not use towel and a few dairy owners used a single towel for all cows commonly to dry the udders. The reuse of towel for cleaning and sanitizing may result in recontamination of the udder**.** Since drying was not or insufficiciently practiced, contamination level of milk was becoming higher.

Furthermore, milkers wash their hands at the beginning of milking but did not dry their hands and not repeat washings between milkings and some of the milkers used milk to moisten the teats when they became dry in between milkings, which could be additional sources of contamination for milk. Handling of small quantities of milk with a big container is subjected to a high rate of contamination with a small milk volume to container ratio (Bonfoh, 2003). This also contributes to the high level of contamination of milk.

In most countries where the risk of contamination during milking, transporting, storage etc is low, the TAPC of raw milk is also low (Gebrhadt and Nicholes, 1975). It was indicated that the TAPC in milk in developed countries falls between $7.5x10^5$ to $2x10^5$ cfu/ml (Gebrhadt and Nicholes, 1975). This value is about 10^2 times and 10^4 times lower than the minimum and maximum values, respectively, recorded for raw milk upon arrival at the processing plant in this study.

According to American and European community member states, the acceptable limit for TAPC and coliform counts for raw milk are between $2x10^5$ and $4x10^5$ cfu/ml and 150 cfu/ml, respectively (APHA, 1992; Heeschen, 1997). However, 10.4% of the milk samples from udder, 45.4% from milking bucket and 100% from storage container and up on arrival at

processing plant had TAPC greater than $4x10^5$ cfu/ml in this study. In the case of coliform counts, 37.7% of the raw milk samples from udder, 96.1% from bucket and 100% from storage containers and upon arrival at processing plant had counts greater than the acceptable limit for America and European countries.

When the mean total aerobic plate counts (5.67×10^9 cfu/ml) and coliform counts (1.26×10^7 cfu/ml) of raw milk upon arrival at the processing plant obtained in this study was compared with the previous reports, the TAPC was higher than that of Mehari (1988) (10^7-10^9 cfu/ml), Hailu (1989) ($1.7x10^7$-$7.5x10^7$ cfu/ml), Rai and Dewvedi (1990) ($4.8x10^6$ cfu/ml), Ashenafi and Beyene (1994) ($2.1x10^6$ cfu/ml), Ombui *et al.,* (1995) (10^5 cfu/ml), DeGraaf *et al*.(1997) ($3.88x10^7$ cfu/ml), Godifay and Molla (2000) ($1.9x10^8$ cfu/ml), Bonfoh (2003) (10^7 cfu/ml) and Esther *et al*. (2004) (10^6 and $3x10^7$ cfu/ml). The mean coliform counts of raw milk in this study was higher than the reports of Rai and Dawvedi (1990) from India ($7.7x10^5$ cfu/ml), Kurwijilla *et al.* (1992) from Tanzania (10^5 cfu/ml), Ombui *et al.* (1995) from Kenya ($5x10^4$cfu/ml), Godifay and Molla (2000) from Ethiopia ($7x10^4$cfu/ml) and Bonfoh (2003) from Mali (10^6 cfu/ml).

The higher values found in this study for both TAPC and coliform counts as compared to the previous reports and EU and United States of America standards were attributed to the cumulative results of milk contamination at different levels while milk was passing through the different critical moments. Factors that could contribute a lot to the contamination of milk in this study include insufficient pre-milking udder preparation, insufficient cleaning of milkers' hands and milking utensils, use of poor quality and non-boiled water for cleaning of udder, milk equipments and storage containers; additional handling of milk into different plastic containers and sieves may cause the contamination of milk higher, since as the number of plastic containers and sieves increased the chance of contamination is also increased and most plastic containers have characteristics that make them unsuitable for milk handling. Since plastic containers scratch easily and provide hiding places for bacteria during cleaning and sanitization and plastic containers are poor conductor of heat and hence will hinder effective sanitization by heat (Shalo, 1990); the proximity of milk collection centers to the main roads which would expose milk to contamination by dust particles and lack of cold chain facility from the initial point of production to upon arrival to the milk processing plant.

In this study udder milk, had a better bacteriological quality because it was not subjected to further contamination after milking. The continuous and progressive contamination of raw

milk as the milk passed through the critical moments was evidenced by the highly significant increment of both TAPC and coliform counts from the initial point of milk production to upon arrival at the milk processing plant (Table 4 and 5). The first contamination occurred up on milking due to insufficient udder preparations, followed by contaminations from milking bucket and milkers' hand at farm level. In addition, contamination of milk occurred as the milk was transported to the milk collection centers and handled at the milk collection centers due to lack of cold chain, use of milk containers lacking tight sealing, handling of milk with unclean hands and utensils and proximity of collection centers to roads. Further contamination occurred as milk was transported to the processing plant without any cold chain facility and using containers, which were not airtight. Bacterial growth could not be considered as the possible cause of increment in TAPC and coliform counts at the critical points of milking bucket at the farm and storage containers at the collection centers since a relatively short time elapsed between these moments (Table 7) and because of the existence of bacteriostatic compounds in fresh raw milk that would counteract bacterial growth for 3 hours (DelloCastillo, 1990; Kurwijilla *et al*., 1992; O'Connor, 1995; Godifay and Molla, 2000). However, the high TAPC and coliform counts at the moment upon arrival at milk processing plant is assumed to be caused by bacterial growth supported by the long time elapsed since the initial point of production (average of four hours) (Table 7) and lack of refrigeration facility through out the critical moments (Kurwijilla *et al*., 1992; O'Connor, 1995; Godifay and Molla, 2000). Under tropical circumstances, high cfu counts of milk delivered at milk processing plants are more often encountered in small holder dairy cooperative (IDF, 1986), even the cfu count observed in this study has to be considered too high. Of course with slight effort in udder and milk equipment hygiene and by increasing the awareness of milk handlers about milk hygiene, the TAPC and coliform count could be reduced at various points of sampling significantly.

The bacterial counts and types of bacteria determine the keeping quality of milk, resulting milk products after processing. Contamination of raw milk in the order of 10^2 to 10^3 cfu/ml is practically unavoidable. Adverse effects in milk start to become apparent when the total count approaches 10^6 and 10^7 cfu/ml (Ombui *et al*., 1995). Milk samples of 4% (udder), 44% (bucket) and 100% (storage container and upon arrival at processing plant) in this study were higher than 10^6 cfu/ml were affecting the quality of milk.

Pasteurized milk in this study had TAPC ranging from 2.65×10^3 to 7.2×10^5 cfu/ml (mean= 2.84×10^4) and coliform counts ranging from 0 to 7.5×10^3 cfu/ml (mean= 1.92×10^2 cfu/ml).

The mean TAPC was compared with the previous reports of Mehari (1988) ($6x10^4$ to $3x10^6$) for Addis Ababa, Sholla dairy processing plant and Esther *et al*. (2004) ($7x10^3$ and 10^4 cfu/ml from two processing plants) in Gaborone and other parts of Botswana dairy processing plants. The result of this finding was lower than that of Mehari (1988). This might be because of pasteurization efficiency variations between the new plant (Mama Milk Processing Plant) and the old (Sholla Dairy Processing Plant) respectively. However, this result was higher than Esther *et al* (2004), because the raw milk bacterial load in this study was much higher by 10^3 cfu/ml. The EU standards, Saskatchewan (1997) recommended that pasteurized milk should not contain total aerobic plate count (TAPC) not greater than $3x10^4$ cfu/ml and while ISO (2000) recommends a non-fecal coliform counts not exceeding 10 cfu/ml and a 0 count in fecal coliforms. Faecal coliform organisms were not encountered in pasteurized milk in this study; therefore, ISO (2000) description of non-coliform count for comparison purpose was taken. Accordingly 7% (TAPC) and 27% (coliform counts) of samples from pasteurized milk did not fulfill the EU and ISO standards (Figure 4). This might be due to post pasteurization contamination and also high bacterial load before pasteurization.

Ashenafi and Beyene (1994) indicated that microorganisms could survive pasteurization temperature if there is high level of contamination of raw milk and therefore keeping such pasteurized milk at room temperature for several hours in retail shops or households may lead to early spoilage of milk (Ashenafi and Beyene, 1994). Therefore, the production of quality dairy products begins at farm level and continues through processing to proper handling of the product by the consumer. Milk quality problems originating on the farm cannot be totally eliminated by processing. Infact some of the quality problems may continue or be magnified during processing and the milk has reached a finished product.

It is not only the bacterial counts, which affect the hygienic quality of milk but also the type of bacteria. Nine percent of the raw pooled milk samples collected directly from the cows' udder was agent free. However, all samples collected from bucket, storage containers and up on arrival at processing plant contained bacterial agents, which were consistently in larger numbers and the isolates types, were more than those from the udders. It has been mentioned that high bacterial counts are indicative of an elevated number of psychrotrophic bacteria (*pseudomonas*, *Flavobacterium* and *Achromobacter spp*), which in turn are responsible for an elevated production of protease and lipase (DeGraaf *et al*., 1997). Even though most emphasis was placed on the high bacterial counts, that psychrotrophic bacterium was present in high numbers. Psychrotrophic bacteria are important because, although mostly not thermoduric,

many of them produce extacellular thermostable proteolytic and lipolytic enzymes which can survive pasteurization (DeGraaf *et al*., 1997), thus affecting the shelf life and quality of the dairy product. In this study psychrotrophic bacterial isolates (*Pseudomonas*, *Flavobacterium*) accounted 5.2% of isolates from udder, 6.5% of isolates from bucket, 6% of isolates from storage containers and upon arrival at processing plant and non of the samples from pasteurized milk.(Table 8). Even though the isolation rate of these bacteria was low, this might contribute to the high bacterial counts to all sampling points of raw milk samples.

In the course of this study bacteria belonging to 17 genera from raw milk upon arrival at processing plant and 8 genera from pasteurized milk were isolated (Table 8). The most predominant genera in raw milk were: S*taphylococcus* spp. (22.8%), *Streptococcus* spp. (21%), *Corynebacterium* spp. (11.7%), *Enterobactr* spp. (6.4%), *Bacilus* spp. (6.4 %), *Klebsiella* spp. (5.9%), *Escherchia* Spp. (5.9%), *Pseudomonas* spp. (5.3%), *Citrobacter* spp. (3.5%), *Enterococcus* spp. (2.9%), *Actinomyces* sp*p.* (1.8%), *Rhodococcus* (1.8%), *Micrococcus spp.* (1.2%), *Serratia* spp (1.2%), *Proteus* spp. (1.2%), *Flavobacterium* spp. (0.6 %) and *Pasteurella* spp. (0.6%). The most predominant genera in pasteurized milk were: *staphylococcus* (45.5%), *Corynebacterium* (33.8%), *Streptococcus* (6.5%), *Enterococcus spp*. (6.5%), *Bacillus* (5.2%), *Actinomyces* (5.2%), *Klebsiella* (3.2%) and *Proteus* spp (0.7%). Even though the isolation rates of *Staphylococcus* spp, *Corynebacterium* spp and *Actinomyces s*pp of pasteurized milk seems higher than raw milk, the percentage of contaminated samples of raw milk with different bacterial isolates is much higher than pasteurized milk.

The results of screening of study animals for mastitis using CMT grades according to Miller and Kearnes (1967) indicated that 76% of the samples were negative while 24% were positive for mastitis. Mastitis infection might have contributed a lot to the many bacterial genera and species isolated and large bacterial counts of milk samples found in this study. This may render milk unsuitable for human consumption (Radostitis *et al*., 1994). In case of acute mastitis where contamination symptoms are clear, considerably more bacteria are growing ($>10^6$ cfu/ml) than in the chronic cases (10^3-10^5 cfu/ml) (Sandholm *et al*., 1995).

In this study isolation of *Staphylococcus aureus*, *Staphylococcus hyicus*, *Staphylococcus intermidus,* CNS, *Streptococcus agalactae, Streptococcus dysgalactae, Streptococcus uberis, Streptococcus bovis, Enterococcus fecalis, E. coli, Actinomyces pyogenes, Corynebacterium s*pp, *Bacillus* spp, *Pseudomonas aeroginosa* and *Enterobacter aerogenes* are incriminated as causes of sub clinical and clinical mastitis in the cow (Harding, 1999). Moreover, *Citrobacter*

freundi, Enterobacter aglomerans, Pasteurella multicoda, Flavobacterium spp*, Streptococcus pyogenes, Klebsiella Pneumoniae,* and *Klebsiella oxytoca* from udder milk samples might indicate that these bacterial agents could probably cause mastitis (Harding, 1999). Microorganisms such as *Streptococcus agalactae, Streptococcus dysgalactae, Staphylococci* spp, and *Coynebacterium bovis* are included in the contagious cause of mastitis, while *Streptococcus uberis*, *Streptococcus bovis, Enterococcus fecalis, Streptococcus pyogenes, E. coli, Actinomyces pyogenes, Corynebacterium* sp*p, Bacillus* spp, *Pseudomonas aeroginosa, Citrobacter freundi, Enterobacter aglomerans, Pasteurella multicoda, Flavobacterium spp, Streptococcus pyogenes, Klebsiella pneumoniae and Klebsiella oxytoca* classified as causes of mastitis caused by environmental origin (Bonfoh, 2003). The contribution of mastitis udder in the bacterial quality of cow milk is an established fact and therefore adequate control of mastitis in the dairy herd is one measure that helps to achieve higher returns to the producer and the processor and to enhance the production of high quality dairy products (Harding, 1999).

Commercial pasteurization resulted in the destruction of most common isolates of pathogenic and spoilage bacteria in raw milk (Table 8). However, organisms such as *Staphylococcus intermidus, Staphylococcus epidermidus, Staphylococcus chromogenes, Streptococcus pyogenes, Proteus vulgaris and Klebsiella oxytoca*, which are normally eliminated by efficient pasteurization, were still present in the commercial pasteurized milk samples. Their presence suggests that the commercial pasteurization process was either ineffective and/or post-pasteurization contamination had occurred. The absence of *Enterococcus faecium* in raw milk as compared to rates of isolation in commercial pasteurized milk was indicative of either pos-pasteurization contamination and/or since the sampled raw milk pasteurized with non-sampled raw milk, this bacterium might be present in-non sampled raw milk. The bacterium thermoduric nature might justify its survival in the pasteurization temperature, as indicated by shearer (2003). *Bacilli* spp isolated in pasteurized milk due to their thermoduric nature. The presence of coliform organisms in pasteurized milk is not an indication of inadequate pasteurization. Instead, it results from some sources after pasteurization (Hagstad and Hubbert, 1986).

The type and number of bacteria present in the milk influence the hygienic quality of milk. Those isolates of *Bacillus* spp, *Staphylococcus* spp, *Micrococcus* spp, *Pseudomonas* spp, *Flavobacterium* spp, *Streptococcus* spp and coliform microorganisms can cause spoilage of the milk when present in raw and pasteurized milk (Doyle, 1997).

From culture positive samples, about 15.6% of samples from udder, 26 % of samples from bucket and 75% of samples from storage container and up on arrival at processing plant were containing *Staphylococcus aureus*. This may be of concern for human health since some strains of *S. aureus* are capable of producing heat stable enterotoxins (Asperger, 1994). However, compared with data obtained from a similar study in Costa Rica, where 8% of samples from udder, 28% of samples from storage containers (Adesiyun *et al.*, 1995) and in Trinidad where 95% of the samples from upon arrival at processing plant were contaminated with *S. aureus*. (Adesiyun *et al*., 1995). The level found in the present study was relatively high compared to data of Costa Rica and relatively low compared to that of Trinidad.

The presence of *Streptococcus agalactae* (Becker, 1994), *Streptococcus pyogenes* (Quinn *et al*., 1999), *Corynebacterium haemolyticum* and *Corynebacterium ulcerans* (Collee, 1989) from the udder, milking bucket, storage container and upon arrival at the processing plant might indicate that their public health significance.

3.9 % of samples from udder, 23% of samples from bucket and 83% of samples from storage container and upon arrival at processing plant contained *E. coli.* Although *E.coli* is frequently occurring organisms in milk and its products, the incidence of *E. coli* itself in milk and milk products as a possible cause of disease is insufficient because *E. coli* normally is ubiquitous organism (Hahn, 1996). Important, however, is the occurrence of pathogenic strains of *E.coli* in milk products, which could be hazardous for consumers.

It was found that in this study 35 (45.4%) of the water samples were proved to be good quality whose coliform most probable number was $\leq$ 3 and the other 42 (54.6%) water samples were poor/non-drinkable quality whose coliform most probable number was $\geq$ 4 according to WHO standards (Figure 5). The presence of coliform bacteria may indicate pollution of water by sewage and the possible presence of pathogenic bacteria (O'Connor, 1995). As sampling time advances, the quality of water also decreased indicating lack of maintenance of the old water pipeline systems. The bacteria flora of most water supplies in the tropics consists mainly of Gram negative-rods. Many of these may be proteolytic and lipolytic and potentially would cause spoilage of milk and milk products if processing and storage conditions were not correct. Water of good bacteriological quality is important to protect human health to avoid milk contamination.

6. CONCLUSION AND RECOMMENDATIONS

Milk intended for human consumption must be free from pathogens and must, if conditions permit, contain no or few bacteria. Clean milk could only be obtained if effective sanitary measures are taken starting from the point of milk withdrawn from the cow until it reaches the consumers. The shelf life of milk be it raw or pasteurized is only increased if the total microbial load in the milk is low at the start of critical points. In line with this fact, the quality of milk produced and channeled to the processing plant from the smallholder dairy cooperative in Debre Zeit area was substandard and the health of dairy herd, hygienic conditions of milking and storage processes, transferring of milk into different containers and sieves, unclean milk equipment and the use of contaminated water were basic determinants of milk quality. Furthermore, the milk was also subjected to more contamination as it was transported to long distances to the processing plant under high ambient temperature and without cold chain facility and using equipments, which were not airtight.

Results from sampling at cntical control points showed that severe contamination started from the first moment the milk left the udder. The difference between TAPC and coliform counts and bacterial isolates at critical control points clearly demonstrated exogenous sources of milk bacterial contamination. This occurred primary at very initial phase of the milking procedure.

Majority of raw milk samples from the bucket and all the samples from storage container and upon arrival at the processing plant had higher TAPC and coliform counts, which was higher than the international acceptable limits. Of the pasteurized milk samples 7% of TAPC and 23% of coliform counts were higher than that of the international acceptable limits. Hence its keeping quality would be lower and some of the pathogens present in the milk have public health significance.

The results obtained in this study showed that raw milk available to the consumers and available to the processing plant has a high bacterial level of contamination. Measurable increased in TAPC and coliform counts through out all sampling points was indicated. Based on the high level of counts found in the milk upon delivery at the plant, one may suppose that this milk may pose a public health risk and this suggests the need for more strict preventive measures.

Based on the findings of the present study the following recommendations are made:

- Awareness should be created among dairy cow owners as to the importance of adequate udder preparation, hygienic milking technique, use of clean dairy equipment, washing of utensils and milkers hands using properly treated water in improve the milk hygienic quality and shelf life.

- Efficient milk cooling system at affordable prices is required not only at the farm level but also at the collection centers and during transportation. Since there is a long time interval between milking and delivery at the plant.

- Raw milk intended for consumption should be subjected to heat treatment at least equivalent to pasteurization temperature.

- If possible, potable water should be available for effective cleaning and sanitizing of milk equipment and udder preparations, otherwise very well heat treated water should be used for such purposes.

- The pasteurization chain and the bottling systems of the processing plant should be regularly monitored to find out the possible source of recontamination or factors which lead to under pasteurization and to take appropriate measures to alleviate the problems.

- Regulation of the dairy development enterprise, establishment of standards and the use of effective enforcement are essential in order to fulfill the consumers' expectations of safe and wholesome milk and milk products

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8. ANNEXES

Annex 1: Questionnaire

1. Description of the farm
   -Name
   -Kebelle
2. Herd size and composition
   -Species
   -Age
   -Herd size
   -Breed: percentage of exotic blood level (0%, 25%, 50%, 75%, >75%)
3. Feeding regime
   -Grazing only/pasture based
   -Stall-feeding: Hay (kg)
   : Straw (kg)
   : Type of concentrate and amount allowed for each cow (kg)
4. Sources of water for the farm
   -Pipeline water
   -Well
   -River
   -Others (specify)
5. Barn hygiene
   -Barn cleaning frequency
   -Bedding condition
6. Pre- milking udder preparation and hygiene
   A. Are flanks, udder or teats washed before milking? Yes/no
   If yes, udder or teat cleaning material
   -Cold tap water
   -Warm water
   -Detergent
   -Disinfectant
   -A combination of the listed options
   -Other (specify)

B. How are teats or udder washed and dried?

C. Do you use teat dips? Yes/no

If yes, when? With what?

-Pre-milking

-Post-milking

-Both before and after milking

7. Milk utensils

A. Do you use bactericidal treatment? Yes/no

B. How often is it cleaned?

-After each usage using cold water

-After each usage using warm water

-Others (specify)

8. Milking technique, milk cooling and transport

A/ Milking procedure used: hand/machine

B/ Milking frequency per day: once/twice/three or more times

C. Do milker's wash hands before milking? Yes/no

If yes, hand cleaning material:

-Tap water

-Detergent

-A combination one of more of the options

D. Are hands washed between cows? Yes/no

-Hand drying

-Yes

-No

E. Average number of cows milked by an individual

F. Milker's clothing

-Clean outer garment

-Own cloth

-Others (specify)

-Interviewer observation

G. How is milk cooled?

-Do you use cooling system for milk? If yes how?

H. Where does the milk go?

-To household consumption

-To processing plants

-To local consumers

-To restaurants

-Others (specify)

I. When does the processing plant take the milk?

-Every day

-Every other day

-Other (specify)

J. Type of storage container used to transport milk to the processing plant

-Aluminum cans

-Plastic bags

-Tanks

-Others (specify)

9. Calf management

A. Hand feeding/bucket feeding

B. Suckling

C. Mixed

10. Calf hygiene

11. Public health aspects

A. Are there tests for tuberculosis? If yes, frequency of the tests.

B. Are there tests for brucellosis? If yes, frequency of the tests

C. Are there screening tests for mastitis? If yes, frequency of the tests

D. Habit of raw milk consumption: yes/no

E. Why illness/symptoms observed in milkers, family members etc.

Annex 2: Split plot experimental design

Block (Date of sampling)	Cc	Critical points			
		A	B	C	D
1	1				
	2				
	3				
	4				
2	1				
	2				
	3				
	4				
3	1				
	2				
	3				
	4				

Cc= collection centers, A= udder, B= bucket, C=storage container, D= upon arrival at processing plant, collection centers = plots, Critical control points =subplots, Date of sampling =Blocks.

Annex 3: Differential tests used for *Staphylococcus*

Ser no.	*Staphylococcus* spp	1	2	3	Fermentation of sugar		
					Manitol	Maltose	Trehalose
1	*Staph. aureus*	+	+	+	+	+	+
2	*Staph. intermidus*	+	-	+	d	(+)	+
3	*Staph. hyicus*	-	-	+	-	-	+
4	*Staph. epidermidus*	d	-	-	-	+	-
5	*Staph. chromogenes*	-	+	-	d	d	NT
6	*Staph. lentus*	-	d	-	+	d	NT
7	*Staph. simulans*	d	-	-	+	(+)	NT
8	*Staph. saprophyticus*	-	-	-	d	+	-

1=hemolysis, 2= pigment production, 3=coagulase test, NT= not tested, d=11-89% strains are positive, (+) =76-89% are positive, (+) = 90% or more strains are weakly positive, + = 90% or more strains are positive, - =90% or more strains are negative.

Source: Quinn, *et al*., 1999

Annex 4: Differential tests used for S*treptococcus*

Ser no	*Strepto coccuss* spp	1	2	3	Sugar fermentation			
					Sorbitol	Manitol	Salcin	Raffinose
1	*Strep. agalactae*	+	-	-	-	-	(+)	-
2	*Strep. dysgalactae*	-	-	-	-	-	-	-
3	*Strep. bovis*	-	-	-	-	V	+	+
4	*Strep. pyogenes*	-	-	-	-	V	+	-
5	*Strep. uberis*	(+)	+	-	+	+	+	-
6	*Strep. zooepidermicus*	-	-	-	+	-	+	-
7	*Enter fecalis*	-	+	+	+	+	+	-

1=CAMP test, 2=Asculin hydrolysis on Edward media, 3=growth on MacConky agar,(+)= majority of strains are positive, v = variable reactions.

Source: Quinn, *et al*., 1999

Annex 5: Differential tests used for C*orynebacterium* and *Actinomyces* spp

Ser.no.	*Corynebacterium* and *Actinomyces*	1	2	Sugar fermentation			
				Glucose	Lactose	Maltose	Trehalose
1	*C. ulcerans*	+	V	+	-	+	+
2	*C. bovis*	+	-	-	-	-	-
3	*C. haemolyticum*	+	-	+	+	+	-
4	*Act. pyogenes*	-	+	+	+	+	V

1=catalase test, 2=haemolysis, v = variable reaction.

Source: Carter (1984)

Annex 6. Differential tests used for *Bacillus* spp

Ser.no	*Bacilus* spp.	Citrate	Arabinose	Manitol	Voges proskauer
1	*Bac. Steariothermophilus*	-	V	-	-
2	*Bac. cerus*	+	-	-	+
3	*Bac. pumilus*	+	+	+	+
4	*Bac. brevis*	d	-	d	-
5	*Bac. coagulans*	d	d	d	d

V = variable reaction ,d = 11-89% strains are positive.

Source: Carter (1984)

Annex 7: Differential tests for Gram-negative rods

Ser. no.	Gram-negative bacteria	1	2	3	4	5	6	7	8	9
1	*E. coli*	+	+	-	-	(+)	-	Y/Y,Gas^+, H_25^-	-	+
2	*Kleb. pneumonae*	-	(-)	+	+	+	+	Y/Y,Gas^+, H_25^-	-	+
3	*Kleb. oxytoca*	+	-	+	+	+	+	Y/Y,Gas^+, H_25^-	-	+
4	*Citrobacter freundi*	-	+	-	+	-	+	Y/Y,Gas^+, H_25^+	-	+
5	*Pseud.aeroginossa*	-	-	-	+	-	-	R/R,Gas^+, H_25^-	+	+
6	*Seratia* spp.	-	-	+	+	+	-	Y/Y,Gas^+, H_25^-	-	+
7	*Flavobacterium*	+	NT	NT	-	NT	NT	NT	+	+
8	*Proteus mirablis*	-	+	-	±	-	+	R/Y,G^+,H_25^+	-	+
9	*Proteus vulgars*	+	+	-	D	-	+	Y/Y,G^+,H_25^+	-	+
10	*Ent. aglomerans*	+	-	+	+	-	D	Y/Y,Gas^+, H_25^-	-	+
11	*Ent. aerogenes*	-	-	±	d	+	-	Y/Y,Gas^+, H_25^-	-	+
12	*Past. multicoda*	+	-	-		-	-	H_25^+	±	-

1 = Indole test, 2 = Methyl red test, 3 = Voges proskeur test, 4 = citrate utilization, 5 = Lysine decarboxylase test, 6 = Urease test, 7 = TSI test, 8 = Oxidase test, 9= Growth on MacConkey Agar, NT=not tested, D=26-75% of strains positive, (+)=76-89% of strains positive, (-)=11-25% strains are positive.

Source: (Carter, 1984 and National mastitis council, 1990)

Annex 8: Primary identification of bacterial pathogens

One loopful of milk streaked on blood agar and Mac Conky agar			
Incubation at 37 ^{o}c for 24-48 hours			
Morphology characterization			
Gram stain			
Gram positive		Gram negatives rods	
Cocci	Rods	Growth on Mac Conky agar	No growth on Mac Conky agar
Oxidative,cat+ =*Micrococcus*	Fermentative, cat+/oxidase-=*Coryn.* spp	Oxidative, cat+/oxidase+ =*Pseud.* spp	Oxidative, cat+/oxidase+ =Flavobacterium
Fermentative,cat+ =*Staphylococcus*	Fermentative, cat-/oxidase- =Act. pyogenes	Fermentative cat+, oxidase- =*Past. haemolytica*	Fermentative, cat+/oxidase+ =*Past. spp*.
Fermentative, cat- =*Streptococcus*	Uncreative, cat+/oxidase- =*Rhodococcus*	Fermentative cat+,oxidase- =Entrobacteriacae	
Uncreative, cat+ =*Rhodococcus*		Unreactive, cat+/oxidase+ = *Alcaligenes* spp, *Past. cabali*	

Source: Quinn, *et a*l., 1999.

Annex 9: Media used for isolation and counts of bacteria

Blood agar base,500 g (Merck, Germany):

Composition (g/l): Nutrient substrate (heart extract and peptones) 20.0; sodium chloride 5.0; agar-agar 15.0. pH 6.8 $\pm$ 0.2 at 25^0c.
Preparations: Suspend 40 g in 1 litre of deminiralized water by heating in a boiling water bath or in current of steam and autoclave at 121^0C for 15 minutes. Cool to 45-50^0c, add 5-8% sterile defibrinated sheep blood and mix taking care to avoid bubble formation. Pour into petridishes.

Mac Conkey, 500g (Merck, Germany):

Composition (g/l): Peptone from casein 17.0; peptone from meat 3.0; sodium chloride 5.0; lactose 10.0; bile salt mixture 1.5; neutral red 0.031; crystal violet 0.001; agar-agar 13.5. pH 7.1$\pm$ 0.2.
Preparation: Suspend 50g in 1 litre of deminiralized water by heating in boiling water bath or in a current of steam; autoclave 15 minutes at 121^0C.

Edwards medium (modified) 500g (Oxoid , England):

Composition (g/l):'Lab-Lemco' powder 10.0; peptone 10.0; asculin 1.0; sodium chloride 5.0; crystal violet 0.0013; thallous sulphate 0.3; agar 15.0. pH 7.4$\pm$ 0.2.
Preparation: Suspend 41g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115^0c for 20 minutes. Cool to 50^0C, add 5-7% of sterile bovine or sheep blood, mix well and pour plates.

Nutrient agar, 500g (Oxoid, England):

Composition (g/l):'Lab-Lemco' powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0. pH 7.4$\pm$ 0.2.
Preparation: Suspend 28 g in 1 litre of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121^0C for 15 minutes.

Trypton water, 500gm (Merck Darmstadt, Germany):

Composition (g/l): Peptone from casein 10.0; sodium chloride 5.0. PH 7.0± 0.2

Preparation: Dissolve 15 gm/l, autoclave in 15 min at 121 ^{o}C.

Procedure and interpretation: One milliliter of ether was added to a 5ml portion of a 48 hrs culture grown at 37^{0}C in a peptone water and shaked well and allowed to stand until the ether rises to the top. Gently Kovac's reagent was added down the side of the test tube and the formation of brilliant red ring between the medium and ether was indicative of an indole production.

MR-VP broth (Methyl Red- Voges-prouskauer) (500g), Merck, Germany:

Composition (g/l): Peptone from meat 7.0; D(+) glucose 5.0; tampon phosphate 5.0. pH 6.9± 0.2.

Preparation: Suspend 17 g in 1 litre of deminiralized water; dispense 5 ml portions into tubes and autoclave 15 minutes at 121^{0}C.

Test procedures and interpretation:

Methyl Red test: The bacteria inoculated into 5 ml MR-VP broth media and then incubated for 48 hrs at 37^{0}C. After incubation 5 drops of methyl red solution was added and a positive reaction indicated by a distinct red colour indicating acidity (pH= 4.4-6.0) and a negative reaction indicated by orange to yellow colour.

Voges-Proskauer test: The bacteria inoculated into 1 ml MR-VP broth media and then incubated for 48 hrs at 37^{0}C. After incubation 0.6ml of 5% alpha-naphtol solution was added, and then 0.2 ml of 40% KOH containing 0.3% creatine was added. The solution was shaked well and left for 5-10 minutes. A positive reaction indicated by development of bright pink or eosin red colour indicating acidity (pH= 4.4 to 6.0) and a negative reaction indicated by no colour change.

Simon's citrate agar,500 g (DCM, USA):

Composition (g/l): ammonium dihydrogen phosphate 1.0; dipotassium phosphate 1.0; sodium chloride 5.0; sodium citrate 2.0; magnesium sulphate 0.2; Agar 15.0 and bromothymol blue 0.08. pH 6.9± 0.2

Preparation: dissolve 22.5 g/l, autoclave at 121 ^{o}C, prepare slant agar tubes.
Inoculation: Slants are inoculated with isolates picked from several like colonies. Incubated at 37 ^{o}C for 24 to 48 hours.
Interpretation: Any blue colour is an indication of citrate utilization .

Lysine Decarboxylase Broth 500 g (DIFCO, USA):

Composition (g/l): Bacto peptone 5.0; yeast extract3.0; bacto dextrose1.0; L-lysine 5.0 and bacto bromo cresol purple 0.02. pH 7 $\pm$ 0.2
Preparation: 14 g of the medium was suspended in one liter of distilled water, boiled to dissolve completely and sterilized at 121^{o}C for 15 minutes.

TSI (Triple sugar iron) agar, 500g (DIFCO, USA):

Composition (g/l):'Lab-Lemco' powder 3.0; yeast extract 3.0; peptone 20.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulphate 0.3; phenol red q.s; agar 12.0.
Preparation: suspend 65 g in 1 litre of distilled water. Bring to BOILING dissolve completely. Mix well and distribute. Sterilize by autoclaving at 121 ^{o}C for 15 minutes and then dispense into test tubes. Allow the medium to set in sloped form with a butt 1 inch deep.
Inoculation: Well isolated colonies are picked with a sterile wire. The slant is streaked and the butt is stubbed. Incubate inoculated tubes at 37 ^{o}C for 18 to 24 hours.
Interpretation:
Acid slant/ acid butt (A/A) due to lactose and /or sucrose fermentation.
Alkaline slant and acid butt (K/A) due to dextrose, no lactose fermentation.
Alkaline slant /alkaline butt (K/K) due to no lactose, sucrose or dextrose fermentation.
Black butt due to hydrogen sulfide production
Note: if tubes are incubated longer than 24 hours, false negatives may occur as a result of reversion of the medium to a neutral colour.

OF- basal medium (Oxidation- Fermentation), 500g (Merck, Germany):

Composition (g/l): Peptone from casein 2.0; yeast extract 1.0; sodium chloride 5.0; dipotasssium hydrogen phosphate 0.2; bromothymol blue 0.08; agar-agar 2.5. pH 7.1$\pm$ 0.2.

Preparation: Suspend 11 g in 1 litre of deminiralized water by heating in a boiling water bath or in a current of steam; autoclave 15 minutes at 121^0C; at approximately 50^0C mix in 100 ml/l of a filter sterilized 10% solution of D (+) glucose, lactose or other carbohydrates; dispense into tubes to give depth of approximately 5cm, in half of the tubes immediately overlay the medium with a 1cm layer of sterile paraffin viscous.

Procedure and evaluation: Inoculate one tube with and without a paraffin seal with a pure culture of the microorganism to be examined down to the bottom of the tube by stabbing technique. The organism used for inoculation should be in the logarithmic phase of growth. Incubation at least 48 hours at the optimal incubation temperature.

Interpretation : a yellow colouration in both the open and paraffin sealed tubes signifies fermentative degradation whereas yellow colouration of the open tubes alone indicates that the carbohydrate in question is broken down by oxidation and absence of colour change indicates uncreative.

Phenol red broth base, 500g (Merck, Germany):

Composition (g/l): Phosphate from casein 5.0; peptone from meat 0.5; sodium chloride 5.0; phenol red 0.018. pH 7.4$\pm$ 0.2

Preparation: Suspend 15 g in 1 litre of deminiralized water; dispense into tubes, if necessary autoclave 15 minutes at 121^0C; at less than 60^0C add the reactants (final concentrations 5-10g/l) as sterile solutions.

Testes for Enzymes:

Catalase test: This demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. A drop of 3 % hydrogen peroxide poured on the glass slide and then small amount of the culture to be tested is picked from a nutrient agar with a clean sterile platinium loop or a clean, thin glass rod and this is added into hydrogen peroxide solution held on glass slide. The production of gas bubbles indicates a positive reaction. It occurs almost immediately. A false positive reaction may be obtained if the culture medium contains catalase (ex. blood agar) or if an iron wire loop is used (Collee, 1989).

Oxidase test: This test depends on the presence of oxidases in the bacteria that will catalyse the transport of electrons between electrons donors in the bacteria and a redox dye -

tetramethyl-p-phenylene-diamine. The dye is reduced to a deep purple colour. The dye is used for screening species of *Alcaligenes, Pseudomonas, Flavobacterium and Pasteurella* spp, which give positive reactions and for excluding the Enterobacteriacae, all species of which give negative reactions.

Wet filter paper method: A strip of filter paper is soaked with a little freshly made 1 % solution of the reagent and then at once used by rubbing a speck of culture on it with a platinum loop. A positive reaction is indicated by a dark purple colour appearing with in 10 seconds, a delayed positive reaction by colouration in 10 to 60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds (Collee, 1989; Quinn, *et al.*, 1999).

Urease test, 500g (Merck, Germany): Composition (g/l): Yeast extract 0.1; potassium dihydrogen phosphate 9.1; disodium hydrogen sulphate 9.5; urea 20.0; phenol red 0.01.
Preparation: Dissolve 38.5 g/l and sterilize in 5 minutes in a current of steam under mild condition. Dispense approximately 3 ml into test tubes.
Experimental procedure and evaluation: Inoculate the medium massively with pure culture under investigation.
Incubation: up to 48 hrs. Interpretation: red = urea positive; Yellow = urea negative

Tube coagulase test): 0.5 ml of rabbit plasma is placed in a small test tube. Two drops of a heavy suspension made from the culture on an agar plate in sterile water, are added then incubated at 37^{o}C. A positive test with cloting of the plasma can occur in 2 to 4 hrs. However, many weak coagulase positive strains will coagulate the plasma only after over night incubation.

Violet red bile dextrose agar (VRBD agar), 500 g (for coliform counts), OXOID, England:

Composition (g/l): Peptone from meat 7.0; Yeast extract 3.0; sodium chloride 5.0; D (+) glucose 10.0; bile salt mixture 1.5; neutral red 0.03; crystal violet 0.002; agar-agar 13.0.
Preparation: Dissolve 39.5 g/l, sterilize by boiling for 1 minute, and do not autoclave it. The prepared medium is clear and slightly red.

Plate count agar (for total aerobic plate count), 500g (OXOID, England):

Composition (g/l): peptone from casein 5.0; yeast extract 2.5; D (+) glucose 1.0; agar-agar 14.0.

Preparation: dissolve 22.5 g/l, sterilize by autoclaving at 115^0C for 20 minutes.

Lactose broth (for MPN of coliform in water), 500g (MERCK, Germany):

Composition (g/l): Peptone from gelatin 5.0; meat extract 3.0; lactose 5.0.

Preparation: dissolve 13 g I 1 liter of distilled water, autoclave (15 min at 121 ^{o}C

9. CURRICULUM VITAE

1. Personal Data

Name	Alehegne wubete Yirsaw
Date of Birth	May 30, 1969
Place of Birth	Finoteselam, Gojjam
Martial status	Married
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Government field veterinarian

2. Education Background

1977-1981	Grade 1-7, Tikurwuha elementary and junior secondary school (Jiga)
1982	Grade 8, Finoteselam elementary and junior secondary school
1983-1986	Grade 9-12, Damot secondary school (Finoteselam)
1987-1992	Addis Ababa University; Faculty Veterinary Medicine

3. Professional Experience

Year	Institution	Responsibility
1991-1992	National Tsetse and trypanosomiasis investigation center	Research as undergraduate associate on tsetse control activities
1994-1998	Ministry of Agriculture	Field veterinarian at Estie district / South Gondar
1999-2000	Ministry of Agriculture	Field veterinarian at Libokemkem district/ South Gondar.
2001-2002	Ministry of Agriculture	Field veterinarian at Fogera district / South Gondar
1995 (July-August)	Woreta Agricultural Technical School	Guest teacher for development agents.
1997 (April to July)	Kombolcha Animal Health Technicians.	Guest teacher for animal health technicians.
2002 (April to July)	Kombolcha Animal Health Technicians.	Guest teacher for animal health technicians

4. Languge

Amharic	Mother Tongue
English	Writing and speaking

5. Membership

Member of the Ethiopian Veterinary association.

6. Research activities

1. Efficacy and cost comparison of alphacypermetrin and deltametrin to the control of *Glossina tachinoid.* Addis Ababa University, Faculty of Veterinary Medicine *(*DVM thesis, 1992).

2. Bacteriological quality of bovine milk in small holder dairy farms in Debre Zeit, Ethiopia, Addis Ababa University, Faculty of Veterinary Medicine *(*Msc thesis, 2004).

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