HYGIENIC PRACTICES DURING MILKING AND BACTERIOLOGICAL QUALITY OF THE MILK IN RAW BOVINE BULK MILK IN THE SELECTED MILK COLLECTION CENTERS: SMALLHOLDER DAIRY PROCESSING ETHIOPIA

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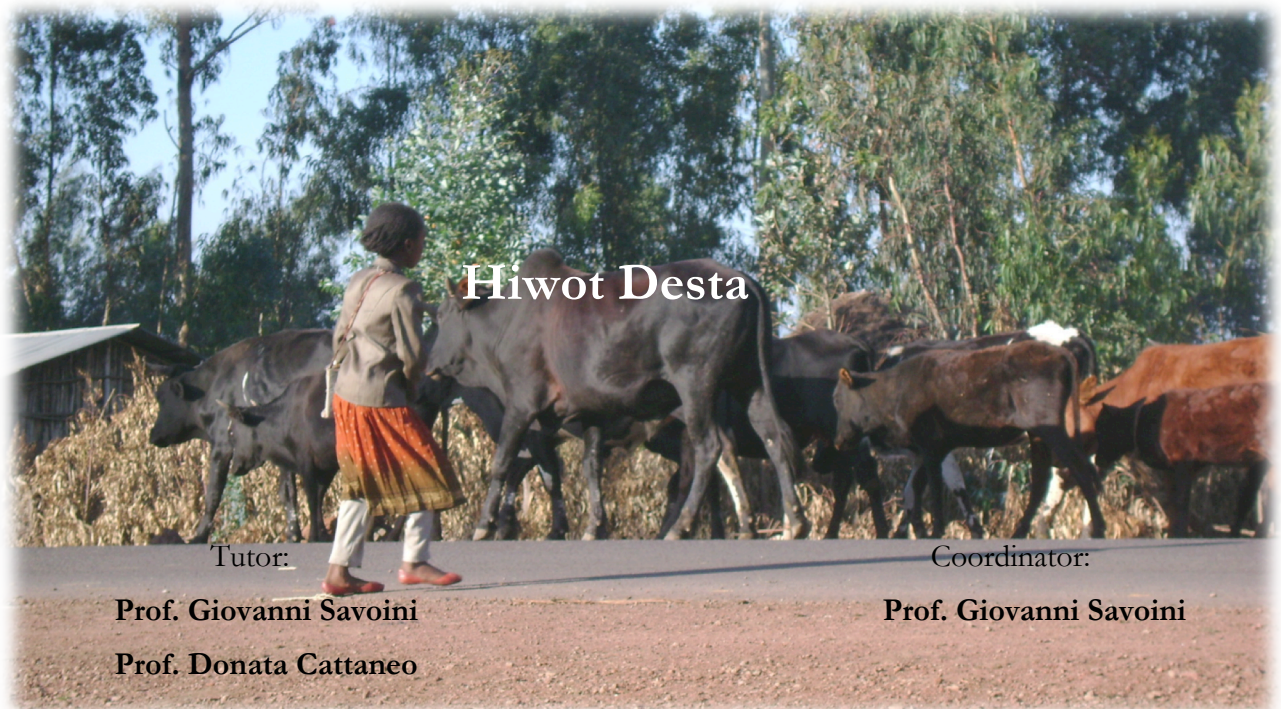
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Academic Year: 2012-2013

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Abbreviations

ADASC	Australian Dairy Authorities' Standards Committee
AEEC	Attaching and Effacing *Escherichia coli*
AFC	Age at First Calving
AI	Artificial Insemination
ANOVA	Analysis of Variance
CA	Community-Acquired
CI	Calving Interval
E. coli	*Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
EMB	Eosin Methylene Blue agar
EPEC	Enteropathogenic *Escherichia coli*
ESAP	Ethiopian Society of Animal Production
ETEC	Enterotoxigenic *Escherichia coli*
FAO	Food and Agricultural Organizations
HA	Hospital-acquired
IACSIT	International Association of Computer Science and Information Technology
ILCA	International Livestock Centre for Africa
ILRI	International Livestock Research Institute
IPCBEE	International Conference on Food Engineering and Biotechnology
IPM	Improving Productivity and Market Success
LA-MRSA	Livestock Associated MRSA
MCC	Milk Collection Centers
MOA	Ministry of Agriculture
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MRT	Milk Ring Test
N	Sample Size
NVI	National Veterinary Institute
OF	Oxidation-Fermentation
PANVAC	Pan African Vaccine Center
PDC	Primary Dairy Cooperatives
RLM	Rapid *Listeria monocytogenes* Agar
SCC	Somatic Cell Count
SD	Standard Deviation
SDDP	Smallholder Dairy Development Project

SDP Smallholder Dairy Project
SE Standard Error
SFP Staphylococcal Food Poisoning
SNV Netherlands Development Organization
SPSS Statistical Package For The Social Sciences
STEC or VTEC Shiga Toxin-Producing *Escherichia coli*
TBC Total Bacterial Count
TSA Trypticase Soy Agar

General Abstract

A cross-sectional study was conducted with a total of 201 member farmers in Arsi Zone and East Shewa Zone, in Oromia, Ethiopia. The purpose of the study was to a assess hygienic practices during milking, milk collection and bacteriological quality of the milk of raw bovine bulk milk in Arsi and East Shewa Zones milk collection centers; the study targeted smallholder dairy processing activities. In Arsi Zones majority of the household heads were male while in East Shewa Zone female were the majority. Main feed used by the respondents was mineral block, oilseed cake, and barn, followed by hay, crop residue, bran, local brewer's yeast and stover\straw and some feed were roughage and/or concentrates. Indoor feeding was common while some used only grazing system. Regarding watering, majority of the respondents, in most cases, used tap water but used river water on rare cases. The average age at first calving in Digeluna Tiyo district is 38.33 months relatively longer than 25.95 months in Tiyo district. The longest calving interval 17.17 months was recorded in Lemuna Bilbilo district, while the shortest was in Tiyo district 14.10 months. The average milk production for local dairy cattle in Tiyo district is 1.45 liters/day, which is less compared to 2.55 liters/day in Lemuna Bilbilo district. The average milk production for crossbred dairy cattle ranged from 5.07 liters/day in Digeluna Tiyo district whereas it was 11.73 liters/day in Tiyo district. Predominantly women do milking. Most of the respondents practiced milking their local and crossed cows twice per day (early in the morning and late in the afternoon) respectively. All the respondents stated that they wash their hands but most respondents wash udder of the cow also before milking. Some of the respondents indicated that they use towel for cleaning the teats before milking the cows. Large proportion of respondents uses plastic as milk container followed by metal and traditional pots. A total of 106 samples of milk were collected to assess bacteriological quality of bulk raw milk. Total of 246 bacterial isolates were obtained from milk samples taken. These included *Escherichia coli* 19.8%, *Listeria monocytogenes* 1.2%, *Staphylococcus aureus* 3.2%and Brucella3.3%. Based on the bacterial isolates found a total of 100 samples of milk were collected to analyzeMethicillin Resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* O157 serotype. Among the total milk samples analyzed for each pathogen, 50.5% were *Staphylococcus* of which *Staphylococcus aureus* were (5.05%), and (47.5%) *Escherichia coli* were also analyzed from the (49.5%) Enterobacteriaceae isolated. Out of the *Staphylococcus aureus* recovered, all the

isolates were sensitive to Methicillin Resistant *Staphylococcus aureus* (MRSA) test and 27.8% of the recovered *Escherichia coli* were positive for E coli O157 serotype.

CHAPTER 1

Introduction

1. Introduction

Ethiopia holds huge potential for dairy development because of its large livestock population, the favorable climate for improved high yielding animal breeds (Ahmed, et al., 2004). Ethiopia's cattle population is estimated at 52.13 million of which 7.2 million are dairy cows and 10.6 milillion are milking cows. The majority of the cattle population is found in the highlands where about 44% of the agricultural human population is residing (CSA, 2012). Livestock and livestock products account for 30% of the agricultural products, and contribute to 46% of the Gross Domestic Product (GDP) (Kelay, 2002). Livestock also contribute at household levels through enhancing income, food security and social status (Eshetu, 2008). Despite this fact, dairying has not been fully exploited and so the direct contribution it makes to the national economy is limited (Ahmed, et al., 2004).

In Ethiopia, the traditional milk production system, which is dominated by indigenous breeds of low genetic potential for milk production, accounts for about 97-98% of the country's total annual milk production (Reda, 1998; Felleke, 2003). Cows are the main source of milk, and as a result, it is cow's milk that is the focus of processing in Ethiopia. However, due to the fact that dairy processing in the country is basically done at the smallholder level, hygienic purities of products are generally poor (Yilma and Faye, 2006). Raw milk is either kept at ambient temperature or kept in a warm place to ferment prior to processing (Moggessie, 2002).

One of the most common food sources in the human diet is milk and is also available to be consumed directly (Grimaud, et al., 2009). Physical, chemical, and biological characteristics of milk favour the multiplication of many bacteria of various genera (Turner and Veary, 1990). This has made the safety of dairy products with respect to food-borne diseases a great concern around the world. This is especially true in developing countries where production of milk and various dairy products take place under rather unsanitary conditions (Yilma and Faye, 2006). Spoilage and contamination of milk and other dairy products may occur as a result of poor hygiene, long periods of transportation without refrigeration services, and lack of appropriate storage facilities (Betre, 2007).

The employment of hygienic practices at the time of milking is, therefore, one of the first and most important steps in clean milk production (Sinha, 2000). In Ethiopia, the consumption of raw milk and its derivatives is common although there is no standard hygienic milking practices, such as personal hygiene of the person who milks, quality of the water used for cleaning purposes and washing the udder before milking, followed by smallholder producers during milk production (Yilma, 2003).

Most of microbial contaminants including human pathogens are members of the family Enterobacteriaceae. Common sources of food contamination by this group of bacteria, especially coliforms that include the genera Escherichia, Klebsiella, Enterobacter and Citrobater, are feces (of animal and human origin), personal hygiene, unclean water and containers (Omore, et al., 2001). *E. coli* 0157:H7 is a newly recognised bacterial zoonosis that originates from the gut of infected cattle. *Escherichia coli* O157: H7 is recognized as a serious cause of diarrhoea, haemorrhagic colitis and haemolytic-uremic syndrome worldwide. Meat, meat products, dairy products, vegetables and drinking water contaminated with animal feces are probably the major sources of the *E. coli* O157: H7 infection (Hajian, at al., 2011). Epidemiological data on *E. coli* 0157:H7 infection and transmission in developing countries remain scarce but it is suspected that consumption of unpasteurised milk is an attention deserving vehicle for its transmission to humans, as milk can easily be contaminated with cattle faeces during milking. Taking into consideration the high proportion of informal sales of unpasteurized milk in many tropical countries, *E. coli* 0157:H7 can be taken as one of the several zoonoses of concern (Arimi, et al., 2000).

Bovine brucellosis is a zoonosis commonly caused by Brucella abortus. The disease in cattle causes abortions and is mainly spread by materials contaminated by body fluids (Seifert, 1996). It results in decreased animal production leading to large economic losses; this is a consequence of abortion, sterility, decreased milk production, reduced reproduction, and the cost of culling animals (Gwida, et al., 2010). The risk of infection by milk-borne brucellosis is one of the reasons for public health regulations, which discourage informal milk markets that sell unpasteurized milk. However, these regulations, despite their importance, are not generally implemented in many developing countries.

Staphylococcus aureus is regularly detected in bulk milk samples as a major contagious pathogen causing intra-mammary infections in dairy cattle (Olde-Riekerink, et al., 2006 and Virgin, et al., 2009). The presence of S. aureus shows up unsanitary conditions in the cattle herd (Tortora, et al., 2005). Milk can be contaminated by S. aureus when there is infection of the mammary gland or bad hygiene during handing and processing, such as not washing hands when handling milk, not washing milk storage equipment during or after milking (Donkor, et al., 2007). Methicillin-resistant S. aureus (MRSA) is a dangerous agent causing both hospital-acquired (haM-RSA) and community-acquired (caMRSA) infections (Enright, et al., 2002; Naimi, et al., 2003). MRSA contaminations were also recovered in dairy foodstuff e.g. bovine milk, mozzarella and pecorino cheeses in addition to meat products (Normanno, et al., 2007).

Listeria monocytogenes   is a pathogenic bacterium that can cause Listeriosis in humans and various animal species (Clark, et al., 2010). The most susceptible domestic species are sheep, goats and cattle. In raw milk and the dairy environment, the source of *Listeria monocytogenes* contamination is mainly from poorly stored silage and unclean bedding materials (Sanaa, et al., 1993; Wagner, et al., 2005). On the farm, contamination of *Listeria monocytogenes* can spread from the environment to the animals and also from animal to animal (Quinn, et al., 2002; Ho, et al., 2007; Barrett, et al., 2005). Contamination of milking equipment with bovine faeces can also occur (Mohammed, et al., 2007). During storage of raw milk on the farm, *Listeria monocytogenes* can grow and multiply, even at refrigerated conditions (Yilma, et al., 2009).

In Ethiopia, in most cases, milk is produced and marketed without any tests for quality (Yilma and Faye, 2006). Information on the microbial and chemical properties of raw milk and milk products produced and marketed by smallholder producers is essential to understand the overall quality of the products being marketed and consumed all over the country.

CHAPTER 2

Literature Review

2. Literature Review

2.1 Overview of the Dairy Sector

2.1.1 *Overview of dairy production systems in Ethiopia*

The different farming systems in Ethiopia vary based on geographical locations and access to markets. They can roughly be categorized into four main systems, although there is variation within each of the systems. The systems are a lowland pastoralist, rural highland smallholder, urban and peri-urban small scale and large-scale dairy production systems.

Lowland pastoralist dairy production system

About 30% of the livestock population in Ethiopia is found in the pastoral areas, these areas comprise 50% of the total land area of the country and have altitudes below 1500 meter above sea level. Pastoralism is the major dairy production system in the lowland. Livestock doesn't provide inputs for crop production but they are the very backbone of their owners providing all of the consumable and saleable outputs, like for example milk, and regarded as insurance against adversity. Milk production is dependent on season due to the rainfall pattern that influences feed availability (Ketema and Tsehay, 1995).

Rural highland smallholder dairy production system

In the highland areas, agricultural production system is predominantly subsistence smallholder mixed farming; crop production is mixed with livestock husbandry. In this system, feed for livestock consists of forages, crop residues, hay, and native pastures grazing. The majority of milking cows in the smallholder milk production are indigenous breeds, which have low production performance (GebreWold, et al., 2000; Ahmed, et al, 2003). The household mainly consumes the milk produced in the traditional system.

Urban and peri-urban small-scale dairy production system

This system is developed in and around major cities and towns located mainly in the highlands of Ethiopia. The main feed resources are agro-industrial byproducts and purchased roughage. The system comprises small and medium sized dairy farmers that own crossbred dairy cows. Farmers use all or part of their land for forage production. The primary objective of milk

production is to generate additional cash income to the household (Ketema and Tsehay, 1995; Azage, et al., 2000).

Large-scale dairy production system

This system is a specialized market oriented dairy operation practiced by the state sector and very few private commercial farms. Most of these farms are located in and around Addis Ababa and basically keep exotic dairy stock (Ketema and Tsehay, 1995; Azage, et al., 2000).

2.1.2   Traditional milk handling and processing practices in Ethiopia

In Ethiopia the smallholder farmers produce fermented milk by traditional methods. The major fermented milk products produced by smallholder farmers by traditional methods include "Ergo" (fermented sour milk), "Kibe" (traditional butter), "Neterkibe" (ghee or clarified butter), "Ayib" (cottage cheese), "Arerra" (sour defatted milk), and "Aguat" (whey) (Ashenafi, 2006).

The fermentation is usually natural, with no defined starter cultures used to initiate it. In most cases, this is made possible through the proliferation of the initial milk flora, with microbial succession determined by ambient temperatures and chemical changes in the fermenting milk. Raw milk is left either at ambient temperatures or kept in a warmer place to ferment. In rural areas, particularly among the pastoralists, raw milk is usually kept in a well-smoked container and milk from a previous fermentation serves as source of inoculums (Ashenafi, 2006).

Ergo (sour milk): is made from raw milk, which is milked in washed and well smoked container (clay pot, calabash) using filtering materials like muslin cloth. Raw milk will be left at ambient temperatures (2-3days) or kept in warmer places to naturally ferment and milk from a previous fermentation serves as inoculum. It is a traditional naturaly fermented milk product, which has some resemblance to yogurt (Mogessie, 2002).

Kibe (traditional butter): is produced by churning ergo in smoked container (clay pot, calabash). The curd is broken by agitation before churning starts. Agitation of churn is carried out by rocking the churn placed on the ground forwards and backwards, or by suspending it from a tripod or doorpost or

shaking it on a person's lap (Almaz, et al., 2001). This process results in the formation of fat granules, which will coalesce into larger grains towards the end of the churning time (1 to 4 hours). Final rotating of the churn on its base would lump the fat grains together into Kibe, which is then skimmed off. The Kibe is kneaded in cold water to remove any residual buttermilk.

Ayib (cottage cheese): Ayib is a traditional Ethiopian cottage cheese made from sour milk after the fat is removed by churning. Churning of sour milk is carried out by slowly shaking the contents of the pot until the fat is separated. The fat is then removed and the defatted milk is heated to about 50ºC to 70ºC until a distinct curd mass is formed and floats over the whey (Almaz, et al., 2001). When the curd and the whey separate, the heating is stopped and the contents of the pot are allowed to cool. After draining off the whey using materials such as muslin cloth, the cheese curd (Ayib) is kept in a clean bowl or pot.

Arrera (sour defatted milk): is another byproduct of ergo obtained after removal of kibe after churning. It has a similar color to ergo, but its appearance is slightly smoother and its consistency thinner, although thicker than fresh milk. It has a pleasant odor and taste. It has a thin consistency and basically contains the casein portion of milk (Almaz, et al., 2001).

Aguat (whey): whey is traditionally known as Aguat. This is the liquid part of Arerra after the Ayib is removed. Heating Arrera 50ºC to 70ºC without markedly affecting product composition yields Aguat (Almaz, et al., 2001).

2.1.3 Milk and milk products consumption patterns

According to Getachew and Geda, (2001), most of the total milk produced is used for human consumption in the form of fresh milk, butter, cheese and yogurt while the rest is given to calves and wasted in the process. Direct consumption of raw milk is much frequent and more popular in Ethiopia than consumption of pasteurized milk because it is believed, especially in rural areas, that raw milk and its byproducts have nutritional advantages and better flavor over the pasteurized milk.

The consumption of milk and milk products vary geographically between the highlands and the low lands and level of urbanization. In the lowlands, all

segments of the population consume dairy products while in the highlands major consumers include primarily children and some vulnerable groups of women.

2.2 Milk Production

2.2.1 *Milking environment*

In Ethiopia, especially when done in the traditional way, there is no standard hygienic condition followed by producers during milk production. The hygienic conditions are different according to the production system, adapted practices, level of awareness, and availability of resources. In most of the cases under smallholder condition, the common hygienic measures taken during milk production especially during milking are limited to letting the calf to suckle for few minutes and/or washing the udder before milking (Zelalem, 2012).

2.2.2 *The cow and the milker*

As observed during milking, most dairy producers do not wash the cow's udder before milking. They rather let calves to suckle before milking. Such practice, however, cannot replace washing. Producers use collective towel to clean the udder of two or more milking cows. Such practice, in addition to its effect on milk quality, can lead to transmission of udder health problems and related complications (Zelalem, 2012).

2.3 Common challenges and constraints of dairy production in Ethiopia

2.3.1 *Feed related constraints*

Lack of adequate feed resources as the main constraint to animal production is more pronounced in the mixed crop-livestock dominated highlands as well as in the mid altitude areas of the country where most of the cultivated areas are located. These areas have high human population density that has led to intensive crop production causing continuous conversion of grazing lands to crop production (Aune, et al., 2001).

Mixed farming system was developed because of the beneficial effect resulting from the interrelationship and complementarities between crop and

livestock production. This made the significance of the role of livestock in the mixed farming system more evident. In the mixed crop livestock production system, livestock production ensures food security and income to the farming community since crop harvest is seasonal. However, it is noticeable that lack of grazinland has induced most smallholder farmers to resort to using crop residues as the major feed resources (Daniel, 1988). The availability of crop residues, as stock feed particularly in smallholder livestock production system, is possible as a result of the allocation of more land for crop production (Alemayehu, 1998).

The quality and quantity of feed supply for livestock in highlands and mid altitude areas is subjected to great seasonal variation. Generally, the availability of feed resources to support the performance of a given class of animal is affected by seasonal fluctuation of rainfall, altitude and soil type. An excessive supply of feed during the rainy season is usually followed by deficit in grazing in the following dry season (Alemayehu, 1998).

2.3.2 Genetic performance
Ethiopia is endowed with large livestock population, unlike other African countries; the large cattle population of Ethiopia encompasses relatively limited numbers of exotic dairy cattle and/or their crosses. The lack of suitable breeding stock poses major constraints. Local breeds need to be crossed with exotic high yielding breeds to increase production. However, according to (Kelay, 2002) activities to improve genetic performance for dairy production in Ethiopia were constrained by a number of factors. The same authors further explained that, climatic stress in the form of erratic and inadequate rainfall, low fodder yield, high price for concentrate and susceptibility to a wide variety of serious diseases damagingly affect the productivity of genetically improved dairy cattle, specially the upgraded ones. Poor education and management expertise of farmers have also been hindrances in the implementation and maintenance of genetic improvement programs.

2.3.3 Animal health
One of the major constraints impeding the development of livestock industry in this country is impact of animal diseases. The prevalence of epidemic

diseases adversely affects the productivity of the animals. The great proportion of the lowland area is infested with trypanosomiasis and its vector tsetse fly. Because of this infestation a vast area of fertile land could not be utilized. Disease commonly found in dairy cattle includes bacterial infections such as anthrax, blackleg, brucellosis, dermatitis, haemorrhagic septicemia, infectious foot rot, mastitis, metritis, pneumonia and tuberculosis (Falvey, et al., 1999).

Many of the animal health problems in the country result from the interaction among the technical constraints themselves. For example, poorly fed animals develop low disease resistance. To overcome health problems and boost up the production and productivity of dairy cattle an integrated farm management should be encouraged and selection for genetic resistance to diseases should be sought. Good health care, herd management and disease control programs impact on dairy cow productivity. Examination and diagnosis should be considered for the entire herd not only individual animals (Falvey, et al., 1999).

2.4   Bacteriological Quality of Raw Milk

2.4.1 Quality Measures

Ethiopia, as a developing country, faces many challenges in producing quality products that are safe for consumption. In Ethiopia, there is no standard hygienic condition followed by producers during milk production. The hygienic conditions are different according to the production system, adapted practices, level of awareness, and availability of resources (Yilma, 2003).

Many collection centers, cooperatives, and processing plants implement quality control measures through two different quality tests: a lactometer reading and an alcohol test. The lactometer combined with a thermometer reading determines the specific gravity of the milk to make sure there is no adulteration. The alcohol test determines if the milk has undergone too much fermentation to undergo further heat treatment. Unfortunately, neither of these tests can determine the presence of bacterial pathogens of public health significance.

Milk and dairy products are potential sources of transmission for many food-borne pathogens. Milk can be contaminated with bacteria of both human and animal origin at any stage in the production to consumption process. Pathogenic organisms can be excreted in the milk from an infected animal (preharvest), or the contamination can occur at the time of collection, processing, distribution, and storage (postharvest) (LeJeune and Rajala-Schultz, 2009)

2.5 Contamination of milk and milk products by pathogenic microorganisms

2.5.1 *Escherichia coli, Escherichia coli O157*

- **Characteristics of *Escherichia coli O157***

  Most *Escherichia coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper, et al., 2004). *Escherichia coli* is a gram negative, rod-shaped, flagellated, non-sporulating, and facultative anaerobic bacterium, which belongs to Enterobacteriaceae family. This bacterium is classified into several categories based on its virulence factors such as *Enterotoxigenic E. coli* (ETEC), Attaching and Effacing *E. coli* (AEEC), *Enteropathogenic E. coli* (EPEC), *Enterohemorrhagic E. coli* (EHEC), and Shiga toxin-producing *E. coli* (STEC or VTEC) (Holko, et al., 2006) and (Wang, et al., 2010).

  Escherichia coli are bacteria that normally inhabit the intestines of humans and animals. Most strains are known to be harmless, but several of them can cause mild to serious disease. One strain in particular, named O157:H7, can cause severe diarrhea and in some cases lead to serious complications, even death. O157:H7 is known to be present in the gastrointestinal tract of cattle, mainly dairy calves (Willert, 1978).

  Many animals, including cattle, sheep, and goats are known to harbor *Escherichia coli* O157, however, cattle are most often implicated as the zoonotic source of human infection (Caprioli, et al., 2005). Cattle feces are the major source of food and water contamination (Rangel, et al., 2005). Many outbreaks of *Escherichia coli* O157:H7 are usually associated with foods from these animals or fecal contamination of water and vegetables by these

animals. Raw milk and yoghurt (Morga, et al., 1993) have been implicated in food-borne illnesses caused by *Escherichia coli* O157:H7.

- **Transmission of disease**

Raw foods, particularly those of animal origin, are frequently contaminated with *Escherichia coli*. People are also carriers of this microorganism and can transmit the microorganism to food products through fecal contamination as a result of inadequate hand washing. *Escherichia coli* can be transmitted through a polluted water supply if used to water fresh fruits and vegetables; these foods too will become contaminated.

Transmission usually occurs through consumption of undercooked or contaminated foods of bovine origin, faecal contamination of other food products or direct contact with infected animals. Cattle and sheep are usually recognized as the principal reservoirs responsible for the proliferation of *Escherichia coli* O157:H7. Since infection occurs via fecal-oral route, the numbers shed in feces and susceptibility of the host ultimately determines transmission of the organism (Kiranmayi, et al., 2010).

Many foods and dairy products have acted as vectors (Rangel, et al., 2005) like ground beef hamburgers kebabs, ready-to-eat cold meats including poultry, pork, and beef products. Waterborne outbreaks have been associated with recreational waters (lakes, ponds, and paddling and swimming pools), drinking water (municipal and local, from springs and wells).

Consumption of unpasteurized milk is an important vehicle for its transmission to humans, as milk can easily be contaminated with cattle feces during milking. Given the high proportion of unpasteurized milk sold informally in many tropical countries, *Escherichia coli* 0157:H7 has been one of several concerns of transmitting zoonoses.

- ***Escherichia coli O157* and milk and milk products**

Milk is one of the most common sources of *Escherichia coli* O157:H7 infection and it is mainly due to fecal contamination (Armstrong, et al., 1996). The frequent epidemiologic evidence of milk as a source of human O157:H7 infection suggests the role of mammary gland, as a potent source of infection (Wells, et al., 1991). *Escherichia coli* O157:H7 was isolated from commercially

distributed raw milk (Willian, et al., 1997), pasteurized milk and also from cheese (Mora, et al., 2007). In U.S. up to 10% raw milk samples from bulk tanks on farms were positive for *Escherichia coli* O157:H7 (Padhye and Doyle, 1991).

- **Situation in Ethiopia**

Little is known about the prevalence of this Serogroup in Ethiopia, either in humans or in the animal population or in foods (Tsegaye and Ashenafi, 2005). Studies isolating *Escherichia coli* O157:H7 from meat samples recorded the highest prevalence in beef, followed by lamb and mutton and goat meat. This revealed the presence of *Escherichia coli* O157:H7 in retail raw meats reaching consumers, indicating possible risks of infection to people through the consumption of raw/under-cooked meat or cross-contamination of other food products (Hiko, et al., 2008). Sheep and goats can be potential sources of *E. coli* O157:H7 for human infection in the country (Mersha, et al., 2010).

- **Control and prevention**

Since the infection primarily occurs via faecal route, the preventive measures include food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking chlorine treated water and personnel hygiene measures like washing hands after toilet visits (Kiranmayi, et al., 2010).

2.5.2 Staphylococcus, Staphylococcus aureus
- **Characteristics of *Staphylococcus aureus***

Staphylococcus aureus is commonly found in the nose, throat (and thus on the hands and fingertips) and on the hair and skin of more than 50% of healthy individuals (Bergdoll, 1979; Robert, et al., 2010). Any food, which requires handling in preparation, may therefore easily become contaminated. Infected wounds, lesions, and boils of food handlers may also be sources of contamination, as well as coughing and sneezing by individuals with respiratory infections. *Staphylococcus aureus* also commonly occurs on the skin and hides of animals, and may thus contaminate foods from these animals as a result of cross-contamination during slaughter.

Staphylococcus aureus is responsible for nearly all Staphylococcal food poisoning (SFP) cases throughout the world, which account for a large portion of gastroenteritis in general. SFP is characterized by the rapid onset of abdominal cramps, nausea, vomiting, and diarrhea.

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a major cause of hospital-associated infections (Chambers, 2001). Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause major illness and death and impose serious economic costs on patients and hospitals. It caused a food-borne outbreak when a delicatessen employee prepared coleslaw. Tests concluded that the employee carried the outbreak strain of Methicillin-resistant *Staphylococcus aureus,* which was presumably transferred from a nursing home that the employee frequently visited (Jones, et al., 2002).

- **Transmission of disease**

The major reservoirs of *Staphylococcus aureus* are infected udders, teat canals, and teat lesions, but these bacteria also have been found on teat skin, muzzles, and nostrils. The bacteria are spread to uninfected quarters by teat cup liners, milkers' hands, washcloths, and flies (Petersson-Wolf, et al., 2010).

Methicillin-resistant *Staphylococcus aureus* can be transmitted from person to person, as well as from animals to humans and vice-versa. Transmission usually occurs by direct contact, often via the hands, with colonized or infected people or animals (Lee, 2003; Ferreira, et al., 2011).

Methicillin-resistant *Staphylococcus aureus* transmission has two main forms, Hospital-Acquired (HA) and Community-Acquired (CA). Although, HA Methicillin-resistant *Staphylococcus aureus* *infection* is thoroughly investigated as the major form, CA Methicillin-resistant *Staphylococcus aureus* presently represents an imminent hazard and may have severe consequences (Calfee, et al., 2003).

- ***Staphylococcus aures* and milk and milk products**

In many developing countries, hand milking is the only method used and allows for a large potential of contamination throughout the milking process. Once the milk is contaminated, *Staphylococcus aureus* strains multiply and

produce SEs as byproducts as long as the conditions are appropriate. Most milk in Ethiopia is kept at room temperature and never refrigerated prior to consumption. This gives enterotoxigenic *Staphylococcus aureus* ample time to proliferate and produce an abundance of SEs (Loir et al., 2003).

An important impediment in the control of *Staphylococcus aureus* infections is its tendency to gain resistance to almost all classes of antimicrobial agents, which it is subjected to (Lowy, 2003).

MRSA has recently been identified as an emerging pathogen in livestock and companion animals. It is a common cause of mastitis in dairy cows and it has been isolated from bulk tank milk (Waage, 1999) and (Lee, 2003). Livestock associated MRSA (LA-MRSA), have been found in farmers and animals, especially pigs and calves (Van Loo, et al., 2007) and (Denis, et al., 2009).

- **Situation in Ethiopia**

Studies revealed the prevalence of *Staphylococcus aures* in dairy products in different areas causing mastitis especially in bovine (Mekonnen, et al., 2011). In Ethiopia 98% of the annual milk is produced by subsistence farmers who live in rural areas under unsatisfactory hygiene conditions, where cooling and other facilities needed for dairy industry are not sufficiently owned by the farmers. This makes these types of foods potential carriers of pathogenic microorganisms, such as enterotoxin producing *Staphylococcus* species (Yilma, et al., 2007).

Resistant *Staphylococcus aureus* strains have been isolated from cattle throughout Ethiopia. Studies isolating *Staphylococcus aureus* from bovine mastitic milk show high levels of resistance to ampicillin, penicillin, polymixin B, and streptomycin (Mekonnen, et al., 2005; Getahun, et al., 2008; Abera, et al., 2010; Belayneh, et al. 2013). Considering the large portion of the Ethiopian population that lives in close proximity to their livestock, there is potential for transmission of resistant *Staphylococcus aureus* from livestock to humans through the consumption of milk.

- **Control and prevention**

The most effective ways to prevent new infections are to eliminate conditions that expose teat ends to bacteria and reduce the possibility of spread from cow to cow; effective udder washing and drying, post-milking teat dip and drying, inter-cow hand washing and disinfection in the milking routine will decrease the risk of contamination of milk by *Staphylococcus* species (Sori et al., 2005).

2.5.3 *Listeria*, *Listeria monocytogenes*,

- **Characteristics of *Listeria monocytogenes***

Members of the *Listeria* genus are short rods, facultative anaerobic, Gram positive, not forming spores and capsules, distributed individually and in form of short chains. The genus *Listeria* contains the two pathogenic species *Listeria monocytogenes* and *Listeria ivanovii* and the four apparently apathogenic species *Listeria innocua, Listeria seeligeri, Listeria welshimeri,* and *Listeria grayi* (Schmid, et al., 2005). *L. marthii* and *L. rocourtiae* these two were described in 2009 (Leclercq, et al., 2009 and Graves, et al., 2010) *Listeria* is ubiquitous in nature, occurring in soil, vegetation, and water (Beuchat and Ryu, 1997; Coyle, et al., 1984; Pearson, 1970), and therefore is frequently carried by humans and animals. *Listeria* can survive for long periods in both soil and plant materials. Ingestion of contaminated silage by ruminants has been linked to the occurrence of *Listeria* in milk (Donnelly, 1987).

Listeria monocytogenes is a gram-positive, short rod facultative intracellular pathogen responsible for severe food-borne infections in humans and causes mortality in susceptible populations, such as newborn children, the elderly, and immune-compromised persons (Huang, et al., 2006; Va´zquez-Boland, et al., 2001). This bacterium is thought to be a saprophytic organism living naturally in the plant-soil environment, where it can survive for up to several months, being able to multiply in decaying vegetation but unlikely to multiply in soil (Fenlon, 1985).

Listeria monocytogenes has been also recognized as a food born pathogen (Kaclikova, et al., 2001) that can contaminate dairy products (Menendez, et al., 2001). Its virulent strain can cause a serious disease called listeriosis, particularly in high-risk populations including pregnant women, newborns,

the very old, and people who are immune compromised (Fleming, et al., 1985).

- **Transmission of disease**

Facilities of dairy plants are excellent environment for development and growth of *Listeria monocytogenes considering* high moisture and milk and milk product residues in the equipment used. *Listeria monocytogenes* has been isolated from many locations within dairy farms, including feces (Lyautey, et al., 2007), (Nightingale, et al. 2004) and (Nightingale, et al. 2005), animal drinking water (Latorre, et al., 2009), (Mohammed, et al. 2009) (Nightingale, et al. 2005) feeds or feed components (Mohammed, et al. 2009), (Nightingale, et al. 2004) and milking equipment (Latorre, et al., 2009), (Latorre, et al,. 2010).

Raw or contaminated food is the most common mode of *Listeria monocytogenes* infection in humans; soft cheeses, delicatessen meats and raw and smoked fish are the usual culprits (Ramaswamy, et al., 2007).

- **Situation in Ethiopia**

Information on the status of food-borne listeriosis is very limited both in the veterinary and public health sectors in Ethiopia. Few studies have been done and from among those studies, Molla, et al. (2004) tried to determine the occurrence and distribution of *Listeria monocytogenes* and other *Listeria* species in retail meat and milk products in Addis Ababa. The result was that 32.6% were found to be positive for *Listeria*; *Listeria monocytogenes* was detected in 5.1% of the samples used (Molla, et al., 2004). Similar studies (Gebretsadika, et al., 2011; Mengesha, et al., 2009) also show 21.6% and 26.6% *Listeria* species and 5.4% and 4.8% were *Listeria monocytogenes;* respectively.

- **Control and prevention**

In order to prevent the contamination of milk and dairy products with *Listeria monocytogenes,* it is necessary to focus on hygiene in dairy plant production facilities. Sanitation measures (washing with detergents, disinfection of equipment, floors, draining pipes, walls, cold stores, etc) must be carried out properly. Very important to keep in mind is that disinfectants have to be changed occasionally; because it is proven that over time *Listeria monocytogenes* acquires resistance to certain preparations (Kasalica, et al., 2011).

2.5.4 Brucella

- **Characteristics of Brucella**

Brucella is Gram- negative coccobacilli (short rods), non-spore forming, facultative intracellular bacteria; it lacks capsules or flagella and non-motile (Corbel, 1997, Arenas, et al., 2000).

Species of Brucella include *B. melitensis*, *B. abortus* and *B. suis*. These 3 species are the most important in terms of public health and economics. In cattle, *B. abortus* causes abortions, stillbirths and weak calves; abortions usually occur during the second half of gestation. The placenta may be retained and lactation may be decreased (Lopes, et al., 2010). *Brucella abortus* induces spontaneous abortion in cattle and causes economic and industrial loss. Although brucellosis has been a health hazard for man and domestic animals in many countries, a licensed human Brucella vaccine has not been designed and produced yet (Schurig, et al., 2002).

- **Transmission of disease**

Brucellosis is a zoonosis caused by the bacterial species Brucella spp. Different types of Brucella infect different, primarily, domestic animals, and are reservoirs for human infections: *B. melitensis* infects sheep and goats, *B. abortus* cattle, *B. canis* dogs, and *B. suis* pigs (Abdussalam and Fein, 1976) and (Sakran, et al., 2006).

Brucellosis is transmissible from animals to humans through contaminated milk, raw milk products, meat or direct contact with infected animals. Humans can become infected through direct or indirect contact with infected animals and their birthing products or by consumption of infected animals' products (Lopes, et al., 2010).

- **Situation in Ethiopia**

Brucellosis is endemic among both small ruminant flocks and cattle of Ethiopia. There are several previous reports of its serological prevalence in these animals in different parts of the country. Several studies were conducted in local and crossbreed animals (Yesuf, et al., 2010), (Ashagrie, et al., 2011), (Bekele, et al., 2010), (Haileselassie, et al., 2010).

There have been relatively high seroprevalence reports of brucellosis prevaelence (above 10%) from smallholder dairy farms in central Ethiopia (Kebede, et al., 2008) while most of the studies suggested a low seroprevalence (below 5%) in cattle under crop-livestock mixed farming (Berhe, et al., 2007; Hailemelekot, et al., 2007; Asmare, et al., 2007; Ibrahim, et al., 2010).

▪ **Control and prevention**

An integrated approach between the human and the animal health sector with government and non-governmental institutions as well as individual farmers and cooperatives is essential. Also test and slaughter where feasible (logistically and financially), pre-movement testing program of upgraded animals (with certification), abattoir surveillance, promotion of pasteurization procedures ideally at the dairy cooperatives level, animal segregation on farm level and health communication. Knowledge of the diseases is a crucial step in the development of prevention and control measures (Prilutski, 2010).

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CHAPTER 3

Objectives

3. Objectives

General objective

Assess hygienic practices of smallholder dairy processing activities (during milking and milk collection) and bacteriological quality of raw bovine bulk milk in Arsi and East Shewa Zones of Oromia Region, Ethiopia

Specific objectives

- To assess dairy husbandry practices, hygienic milking and milk handling practices, and constraints of the dairy sector in selected districts of Arsi and East Shewa Zones; Chapter 4

- Study occurrence of *Escherichia coli*, *Listeria monocytogeneses*, *Staphylococcus aureus* and Brucella in raw bulk bovine milk in selected districts of Arsi and East Shewa Zones; Chapter 5

- Study occurrence of Methicillin Resistant *Staphylococcus aureus* and Enterotoxigenic *Escherichia coli* (O157) in raw bovine bulk milk in selected districts of Arsi Zone; Chapter 6

General Methodology

A cross-sectional survey with closed and open-ended questions was used to interview smallholder dary producers to gather information on the following issues:

on Household (HH) characteristics family size, sex, and age of owners; cattle herd composition and herd structure;.

Reproductive and productive performance: age at first calving and calving interval, milk yield;

Hunabadry practices: type of feed, feed source, traditional milking practices, feeding and watering strategy and breeding practices and occurrence of major livestock diseases and availability of veterinary services;

For the survey, 201 smallholder milk producers were selected and interviewed with the help of development agents of the Ministry of Agriculture (MOA) of

each zone. Following proportional allocation of the sample size Tiyo, Digeluna Tijo, and Lemuna Bilbilo districts were selected from Arsi Zone and Ada'a district and Debre Zeit town were selected from East Shewa Zone were selected based on ease of access for logistic reasons; access to milk and milk product market. 106 milk samples from both Arsi and East Shewa Zones were selected for identification of different bacteria and for the second trial 100 milk samples were picked from Arsi Zone for MRSA and Serotype E.coli O157 were proportionally allocated to the selected milk collection centers (MCC) and randomly collected from each district.

Milk Sampling Procedures: Milk samples were collected aseptically and samples were transported to National Veterinary Institute (NVI) laboratory for bacteriological examination in an icebox at temperature below 4°C and were analyzed within 36hrs of collection.

Frozen milk samples were thawed at room temperature. The bacteriological cultures were performed following standard microbiological techniques (Quinn, et al., 1994). For identification of Gram-positive and Gram-negative bacteria one loop of milk was streaked on 5% sheep blood agar and MacConkey agar plate, respectively. The plates were incubated aerobically at 37^0C for 24 hours. Presumptive identification of bacteria on primary culture was done on the basis of colony size, morphology, heamolytic characteristics, gram stain reactions, and catalase test. Catalase test was done on colonies transferred on to nutrient agar.

Data analysis
All the collected data were stored and managed in Microsoft Excel database. The data were analyzed using the statistical software SPSS (Version 16.0, 2006). Data obtained from survey such as herd characteristics, composition and husbandry practices were reported using simple descriptive statistics. Analysis of variance (ANOVA) was used to test the variability of different variables; age at first calving, calving interval, amount of milk produced from different breeds of cattle (cross or local) across the districts. The results for microbial statistical in different primary diary cooperatives were summarized using descriptive statistics (means, standard errors, proportions). Significant differences were considered as (p<0.05).

CHAPTER 4

Cross-Sectional Study on Husbandry and Milking Practices in the selected milk collection centers of Arsi Dairy Union and Ada Dairy Cooperatives in Oromia Region Ethiopia

Result and Discussions

4. Cross-Sectional Study on Husbandry and Milking Practices in the selected milk collection centers of Arsi Dairy Union and Ada Dairy Cooperatives in Oromia Region Ethiopia

4.1 Abstract

A cross-sectional study was conducted to assess hygienic practices during production and subsequent handling of milk by cooperative member farmers in Arsi Zone and East Shewa Zone in Oromia Region in Ethiopia. A total of 201 farmers were interviewed from selected three districts (Tiyo, Digeluna Tijo, and Lemuna Bilbilo) in Arsi Zone and Ada district (Debre Zeit town) in East Shewa Zone. In Arsi Zone 10.0% of the household heads were females and 40.3% were male while in East Shewa Zone 32.3% of the household heads were females and 17.4% were male. From the total respondents, the Orthodox Christians (80.6%) population was higher than the Muslims (11.9%) and Protestants (7.5%) population. Out of the total respondents, 28.9% fed their animals mainly mineral block, oilseed cake, and wheat bran, 20.4% of the respondents fed mineral block, oilseed cake, hay, crop residue, wheat bran, local brewer's yeast and stover\straw while the rest fed roughage and/or concentrates. Majority of respondents used indoor feeding (84.1%) some used only grazing (13.9%) and a few others used a mixture of both systems (2.0%). Regarding watering 61.2% used tap water while 37.8% used river water and 1.0% of the respondents used both water sources. The average age at first calving ranged from 38.33 months in Digeluna Tijo district to 25.95 months in Tiyo district. Differences in age at first calving across different districts were statistically significant ($p<0.05$). The longest calving interval (17.17 months) was recorded in Lemuna Bilbilo district, while the shortest was in Tiyo district (14.10 months). Differences in calving interval across different districts were not statistically significant ($p<0.05$). Average daily milk yield for local dairy cattle ranged from 1.45 liters in Tiyo district to 2.55 liters in Lemuna Bilbilo district. However, these differences in milk yield across the study sites were not statistically significant ($p>0.05$). The average daily milk

yield for crossbred dairy cattle ranged from 5.07 liters in Digeluna Tijo district to 11.73 liters in Tiyo district and these differences across the study areas were statistically significant (p<0.05). Predominantly women (55.7%) do milking while 28.9%, 9%, and 6.5% were done by female and male, male and combination of all household members, respectively. Most of the respondents (99.5% and 97.5%) practiced milking their local and crossed cows twice per day (early in the morning and late in the afternoon), respectively. All the respondents stated that they wash their hands and most respondents (94.5%) wash udder of the cow before milking and 85.6% of the respondents also indicated that they use towel for cleaning the teats before milking cows. Large proportion of respondents (88.1%) used plastic materials as milk container, 8% used metallic materials and 3.5% used traditional pots.

Keywords: Milk, smallholder producers, milking practices, husbandry practices

4.2   Location, Materials and Methods

Study Location

Baseline information gathering was conducted in Arsi Zone and East Shewa Zone. For ease of accessibility as well as for logistic reasons, three districts from Arsi Zone (Tiyo, DigelunaTijo, and LemunaBilbilo) and from East Shewa Zone (Ada district, DebreZeit town) were selected to provide baseline information.

The activities were done in Arsi Zone (Tiyo, DigelunaTijoand LemunaBilbilo Districts) and East Shewa Zone (Ada District, DebreZeit town).
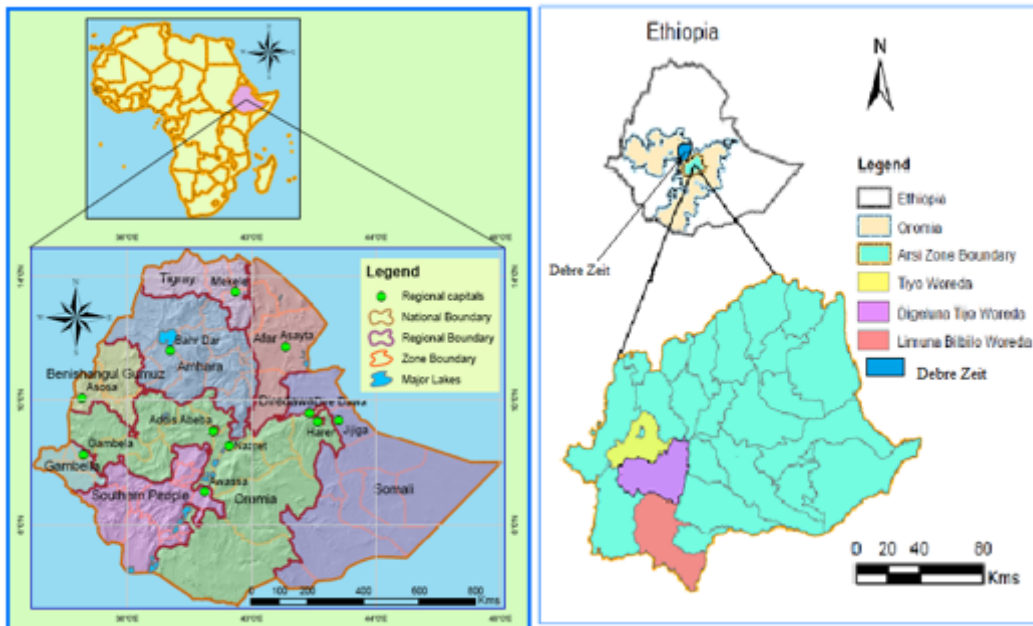
Figure I The geographical location of the study areas

Tiyo district: - is located at about 167 km south east of Addis Ababa and at the foot slopes of Mount Chilalo in the eastern side. Tiyo has a total area of 65,000 hectares of land and from these, 25,060 hectares is used for crop cultivation, 9,697 hectares for grazing, 3,959 hectares for forest, 9,479 hectares is covered by bush and shrub, 10,828 hectares is barren and 5,977 hectares used for other purposes. Tiyo has diverse climatic conditions; weynadega (52%), dega (37%), and kola (11%) agro- ecologies with altitude ranging from below 2300 to over 3200 meters above sea level. Tiyo has got 1300mm to 1350mm annual rainfall and an average temperature of 18°c to 25°c during dry season and 5°c to 10°c during wet season. The area experiences bimodal rainfall, that is long rainy season occurring from June to August and short rainy season from February to April. It has very productive environmental conditions due to its climate and soil. The dominant cereals cultivated in the area are wheat and barley (FAO, 2006).

DigelunaTijo: - is located 192 km from the capital Addis Ababa in the southeast. The administrative center of this district is Sagure. The district has a total area of 92,700 hectares of land and from these, 43,873 hectares is used for crop cultivation, 15,054 hectares for grazing, 11,122 hectares for forest, and 22,651 hectares used for other purposes. The district has diverse climatic conditions; dega (78%) and weynadega (22%) agro-ecologies with altitude ranges from 2500

to 3560 meters above sea level. The area experiences bimodal rainfall, long rainy season occurring from June to September and short rainy season from mid March to April (FAO, 2006).

Lemuna Bilbilo: - is located 223 km from the capital Addis Ababa in the southeast. The administrative center of this district is Bekoji and the district has a total area of 81,400 hectares of land and from these; 70,154 hectares is used for crop cultivation, 6,746 hectares for grazing, 3,839 hectares for forest, 262 hectares of land is covered by bush and shrub, 99 hectares is barren and 300 hectares used for other purposes. The district has diverse climatically conditions; dega (80%) and weynadega (17%) and kola (3%) agro-ecologies with altitude ranges from 2500 to 3560 meters above sea level. The area experiences bimodal rainfall, long rainy season occurring from June to August and short rainy season from mid March to April (FAO, 2006).

DebreZeit: - is located 47.9 kilometres southeast of Addis Ababa. It the largest district in East Shewa zone (1610.56 km^2) bordering Akaki in the west, Gimbichu in the north, Lume in the east, Dugda-Bora in the south and Southern Peoples' Regional State in the south west. DebreZeit (Bishoftu) is the district capital. There are about ten lakes in the district and most of these are crater lakes. About 90% of the district belongs to the sub tropical agro-climatic zone. The tropical and temperate agro-climatic zones cover 5% of the district each. Ada'a Liben is the second populous district in East Shoa zone with a total population of 248,274 in 1997. The urban population accounted for 33.8% of the total population in the district. Ada'a Liben is one of the top district of East Shoa zones in the production of cereal crops such as teff and wheat as well as various types of pulses.

4.3   Data Collection

The approximate sample size required was estimated using the formula given by Arsham, (2005) for survey studies:

N = 0.25/SE^2, Where: SE = Standard error, N = Required sample size
Hence, at 5% standard error, a total number of 100 individual households were selected randomly for the baseline survey from each Zone making a total of 200 households.

4.4   Data collection methods

Questionnaire survey

Semi-structured questionnaire with closed and open-ended questions were the major tools used during the survey phase of the study. The study included data collection on household characteristics, and the cattle herd structure, reproductive performance, production performance, herd management, breeding practices, occurrence of major livestock diseases and availability of veterinary services.

4.5   Data analysis

All the collected data were stored and managed in Microsoft Excel database. The data were analyzed using the statistical software SPSS (Version 16.0, 2006). Data obtained from the survey were reported using simple descriptive statistics. Analysis of variance (ANOVA) was used to test the effect of different factors on selected variables.

4.6   Results

4.6.1   Characteristics of the respondents

Respondent profile

Of the total 201 dairy producers interviewed from the two Zones, 42.3% were female while 57.7% were male. The average age of the respondents was 48 years. In Arsi Zone 10.0% of the household heads were female and 40.3% were male while in East Shewa Zone 32.3% of the household heads were female and 17.4% were male. Most of the respondents (45.8%) were aged between 41-61 years; second largest group aged between 20-40 years (36.8%) and the ones older than 62 years represented only 17.4%. Looked at from the perspective of religion, from the total respondents, the Orthodox (80.6%) population was higher than the Muslim (11.9%) and the Protestant (7.5%) population (Table I).

With regard to family size, out of the total respondents 53.7% have a family size of 6-11 while respondents that have a family size of 1-5 and 12-16 were 42.8% and 3.5%, respectively. In Arsi Zone 26.9% of the respondents have a family size of 6-11 and 19.9% have a family size of 1-5 while 3.5% have a family size of 12-16; in East Shewa Zone 26.9% of the respondents have a family size of 6-11. In the present study, the average dairy farming experience was 14.81 years (Table I).

Table I Characteristics of respondents

Variables	Frequency (%)
Sex	
Female	**85 (42.3)**
Arsi Zone	20 (10)
East Shewa Zone	65 (32.3)
Male	**116 (57.7)**
Arsi Zone	81 (40.3)
East Shewa Zone	35 (17.4)
Total	**201 (100)**
Age (years)	
20-40	**74 (36.8)**
Arsi Zone	41 (20.4)
East Shewa Zone	33(16.4)
41-61	**92 (45.8)**
Arsi Zone	47 (23.4)
East Shewa Zone	45 (22.4)
62-82	**35 (17.4)**
Arsi Zone	13 (6.5)
East Shewa Zone	22 (10.9)
Total	**201 (100)**
Religion	
Orthodox	**162 (80.6)**
Arsi Zone	72 (35.8)
East Shewa Zone	90 (44.8)
Muslim	**24 (11.9)**
Arsi Zone	22 (10.9)
East Shewa Zone	2 (1.0)
Protestant	**15 (7.5)**
Arsi Zone	7 (3.5)
East Shewa Zone	8 (4.0)
Total	**201 (100)**

Family size	
1-5	**86 (42.8)**
Arsi Zone	40 (19.9)
East Shewa Zone	46 (22.9)
6-11	**108 (53.7)**
Arsi Zone	54 (26.8)
East Shewa Zone	54 (26.9)
12-16	**7 (3.5)**
Arsi Zone	7 (3.5)
East Shewa Zone	0 (0.0)
Total	**201 (100)**

Average cattle herd size per household

The cattle herd size and composition of the study districts are summarized in the Table 2. The total cattle populations of different breeds owned by respondents in DebreZeit, DigelunaTiyo, LemunaBilbilo and Tiyo districts were 467, 208, 647 and 228 respectively.

Table II Cattle population and composition

Cattles by Breed	Herd at each district				Total (%)
	Ada (N=100)	Digeluna Tiyo (N=15)	Lemuna Bilbilo (N=46)	Tiyo (N=40)	
Local breeds					
Lactating cows	4	23	58	6	**91 (5.9)**
Dry cows	5	21	57	5	**88 (5.7)**
Oxen/Bulls	42	25	110	70	**247 (15.9)**
Male calves	2	9	61	12	**84 (5.4)**
Female calves	5	13	35	7	**60 (3.9)**
Cross breeds					
Lactating cows	224	42	85	60	**411 (26.5)**
Dry cows	30	12	39	6	**87 (5.6)**
Oxen/Bulls	2	21	91	17	**131 (8.4)**
Male calves	26	18	45	13	**102 (6.6)**
Female calves	127	24	66	32	**249 (16.1)**
Total	**467**	**208**	**647**	**228**	**1550 (100)**

N = Required sample size

4.6.2 Dairy cattle husbandry practices

Feeds and feeding management

Livestock are kept under traditional management conditions in both study areas. Out of the total respondents, 28.9% fed their animals mainly concentrates (oilseed cake, and wheat bran) and mineral block, 20.4% of the respondents fed their animals mainly concentrates (agro-industrial by-products) (oilseed cake and wheat bran), natural pasture (hay and green grass), crop residue (rice, Teff, barley/wheat straw, maize or sorghum Stover), (Stover-the leaves and stalks of corn, or sorghum plants that are left in a field after harvest). Stover can be directly grazed by cattle or dried for use as fodder. It is similar to straw. The remaining large proportion of respondents feed their animals with mainly natural pasture (hay and green grass) and crop residues.

The main feeding systems used in the study areas were indoor feeding and grazing; a mixture of both systems is also used. Majority of respondents used indoor feeding (84.1%) those who used only grazing constituted (13.9%) and a

few (2.0%) used a mixture of both systems. In Arsi Zone, most respondents (35.8%) used indoor feeding, 12.4% used only grazing and 2.0% used both systems while in East Shewa Zone 48.3% used indoor feeding and 1.5% used only grazing.

Wheat strawCorn Stover

Crop residues

Brewery by-productHay

Figure II Feeds

Water source and watering practices

Respondent had two different water sources; these are river and tap water. Majority of the respondents (61.2%) used tap water while 37.8% used river water and 1.0% of the respondents used both water sources. In Arsi Zone 37.8% used river as source of water while 49.8% respondents in East Shewa Zone used tap water as water source.

Among the respondents in the study area, 39.3%, 43.8% and 12.4% provided their animals with water once, twice and three times per day, respectively. Of the respondents 4.5% watered their dairy cattle on Ad libitum basis. In Arsi Zone 25.9% and 21.4% of the respondents gave water to their animals once and twice per day, respectively. In East Shewa Zone 13.4%, 22.4% and 12.4% of the respondents provided water to their animals once, twice and thrice per day, respectively.

Figure III River water source

Reproductive performance

Age at first calving (AFC) and calving interval (CI) were used to assess the reproductive performance. As shown in Table III, the average age at first calving was 38.33 months in Digeluna Tiyo district while it is 25.95 months in Tiyo district. Differences in age at first calving across different districts were statistically significant (p<0.05). 17.17 months calving interval in Lemuna Bilbilo district was recorded as the longest while in Tiyo district 14.10 months was recorded as the shortest. Differences in calving interval across different districts were not statistically significant (p<0.05).

Table III Mean of Age at first calving (AFC) and calving interval (CI)

District	N	Reproductive performance (Mean ± SD)	
		AFC (months) (P=0.000)	CI (months) (P=0.05)
DebreZeit	100	25.97±4.16	14.51±3.93
DigelunaTiyo	15	38.33±9.17	15.20±5.00
LemunaBilbilo	46	35.04±8.03	17.17±5.43
Tiyo	40	25.95±6.51	14.10±4.55
Total	**201**	**28.97±7.65**	**15.09±4.63**

N = Required sample size

4.6.3 Milk production

The primary purpose of milk production for most of the respondents (89%) was for both sale and household consumption. On the other hand, 9.5% and 1.5% of the respondents produce milk only for sale and only for household consumptions, respectively.

The following table presents the average daily milk yield of local and cross breed dairy cows. Average milk yield for local dairy cattle in Tiyo district is 1.45 liters while it is 2.55 liters in Lemuna Bilbilo district. However, these differences in milk production across the study sites were not statistically insignificant (p>0.05). The average daily milk yield for crossbred dairy cattle in Digeluna Tiyo district is 5.07 liters whereas in Tiyo district it is 11.73 liters, and these differences across the study areas were statistically significant (p<0.05).

Table IV Average Milk Production of Local and Crossed breeds

District	Average milk production (Mean ± SD)			
	N	Local (liters/day) (P=0.000)	N	Crossed (liters/day) (P=0.000)
Debre Zeit	100	1.75±0.45	100	10.81±3.75
Digeluna Tiyo	15	2.33±0.97	15	5.07±2.12
Lemuna Bilbilo	46	2.55±1.19	46	6.72±2.90
Tiyo	40	1.45±0.81	40	11.73±5.44
Total	**201**	**1.92±0.88**	**201**	**9.63±4.47**

N = Required sample size

Milking and milk handling practices

Predominantly women or adult females (55.7%) do milking while 28.9%, 9%, and 6.5% were done by female and male, male and combination of all household members, respectively. In Arsi Zone milking predominantly is done by female (32.3%) followed by male and female (11.9%) and a combination of all hosehold memberd (6%). In East Shewa Zone milking predominantly is done by female (23.4%), male and female (16.9%) and a combination of all household members (9.5%).

Figure IV Hand milking by woman and man

Most of the respondents (99.5% for local and 97.5% for crossbred cows) practiced milking cows twice per day (early in the morning and late in the afternoon) respectively. All the respondents stated that they wash their hands before milking and most respondents (94.5%) wash udder of the cow before milking and 85.6% of the respondents also indicated that they use towel for cleaning the teats before milking cows.

Large proportion of respondents (88.1%) use plastic materials as milk container, 8% use metallic materials and 3.5% use traditional pots. Majority of the respondents in Arsi Zone (45.8%) use plastic vessel and 3.5% of the respondents use traditional pots. In East Shewa Zone majority of the respondents (43.3%) use plastic vessel and the rest (7.5%) use metal vessel.

Figure V   Plastic Bucket and jerry cans,   Clay pot,   Aluminum

Milk processing

All the primary dairy cooperatives (PDC) have small scale processing units and have the minimum required facility for milk processing units. All the processing

units have a cream separator and complementary equipment; they also own milk churner for milk processing. They use a hand operated cream separator. The cream is soured and churned using a rotary wooden churn for butter making while the skim milk is soured and cooked for Ayib making. The cream is then soured for butter making, while fermented skim milk is used as a raw material for Ayib making (Figure VI).

Figure VI Traditional butter (Kibe), Ethiopian cottage cheese (Ayib), Traditional yoghurt

Figure VII Hand operated cream separator

At household level, in Arsi Zone 33.8% and in East Shewa Zone 7.5% process milk using traditional method. Most respondents process milk into butter, Ethiopian cottage cheese, and yoghurt. Others process it to Aguat (Figure VI).

Figure VIII flowchart:

Fresh cow's milk

Washed & smoked container (clay pot, calabash)
Filtration using materials like muslin cloth
Natural fermentation at ambient temperature (2 – 4 days)
Addition of additives: rue, garlic

Ergo[1]

Churning using the same container or other pre-washed and smoked container
Addition of warm water

Butter

Buttermilk

Cooking at low temperature (50–70˚C/50-70 min)
Filtration

Cooking at boiling temperature (50-60 min) (clarification)

Nitir kibe[2]

Ayib[3]

Aguat[4]

Figure VIII Traditional milk processing: [1]Naturally fermented cow's milk, [2]ghee (clarified butter), [3]Ethiopian cottage cheese (is mild and crumbly, it has little flavor on its own and is often served as a side dish to soften the effect of very spicy food), and [4]whey (source: Z. Yilma, et al. 2007)

Figure IX flowchart:

Fresh cow's milk

Manual cream separation

Cream

Skim milk

Natural souring at room temperature (2-4 days)

Sour cream

Sour skim milk

Manual churing

Cooking at low temperature (50-70˚C)
Filtration

Butter

Buttermilk

Ayib[1]

Whey
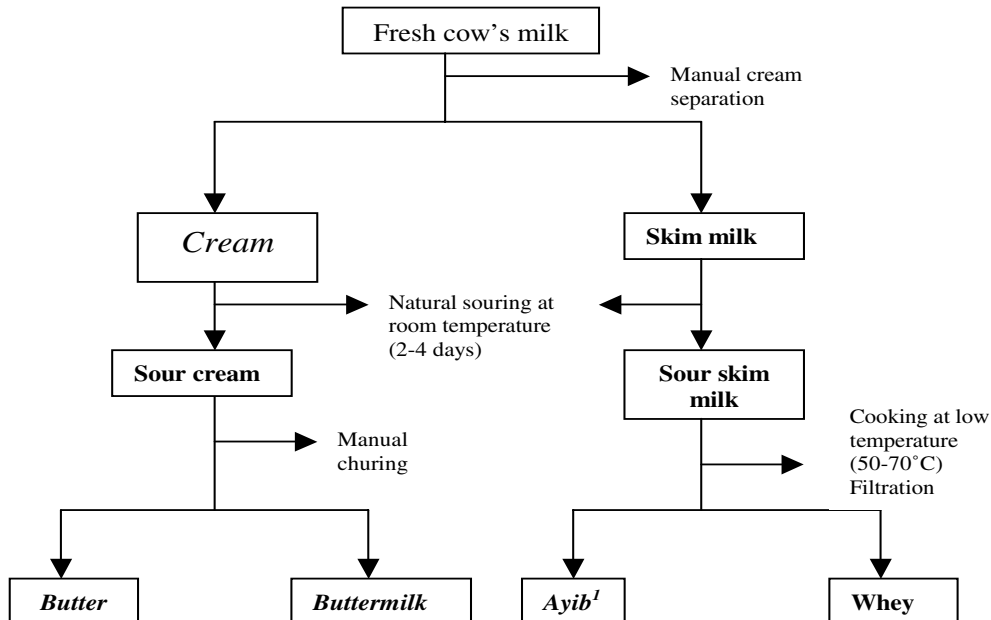
Figure IX Milk processing at cooperatives centers[1]Ethiopian cottage cheese (source: Z. Yilma et al. 2007)

4.6.4 Animal diseases and access to veterinary services

Respondents in both Arsi and East Shewa Zones encounter different animal diseases, namely parasitic, bacterial, reproductive disorder, TB, anthrax, and miscellaneous causes. The animal diseases mentioned by the respodents are Black leg (18.9%), parasites (11.9%), mastitis (7.3%), milk fever (5.5%), and TB (4%). In addition, 8.5% of the respondents replied reproductive disorder is also a common problem.

Out of the total respondents, 98% get access to veterinary services and AI services either through government or private veterinary clinics while the rest didn't get veterinary service either from government or private sectors. Shortage of experts, lack of adequate transport facility and road problem in some parts of the districts and affordability of veterinary drugs and services are some of the problems mentioned by those dairy producers as hindrances to get veterinary and AI services. In Arsi Zone, 33.3% respondents mentioned that their service provider is mostly the government whereas in East Shewa Zone 47.8% of the respondents indicated that the private veterinary clinic is their service provider.

4.6.5 Record keeping

Record keeping was not common in the study areas. Only 37.8% of the total interviewed dairy producers tried to keep some production and reproduction information. 14.4% and 23.4% of the respondents in Arsi and East Shewa Zones keep production and reproduction information, respectively.

4.6.6 Main constraints of the dairy sector

The most important livestock and milk production constraints prioritized by the farmers of the study areas are unavailability and high costs of feeds, poor veterinary services, discouraging seasonal milk marketing opportunities, poor artificial insemination (AI), water scarcity, limitations of land for sustainable dairy development and late payment for the milk sold. The extent and significance of the problems and constraints differed between the different zones. More than 50% of the respondents in Arsi Zone, mentioned the major constraints are unavailability and high costs of feeds, poor veterinary services, discouraging seasonal milk market opportunities, poor artificial insemination, water scarcity and limitations of land for sustainable dairy development. Whereas in East Shewa Zone the major constraints mentioned (35.3%) were unavailability

and high costs of feeds and late payment for the milk sold, followed by other different limitations.

4.7   Discussions

4.7.1   *Characteristics of the respondents*
Sex and religion distribution

The finding on the respondents' sex structure is similar to the reports of Sintayehu, et al. (2008) and Tesfay, et al., (2012) who reported 77% male and 23% female household heads and 81.25% male and 18.75% female household heads, respectively. As for religion, the same authors reported overwhelming majority of Orthodox religion among respondents, which is in line with the present study.

Having more family size per household is likely to be considered as an asset and a factor, which increases social security in times of retirement (Agajie, et al., 2002). Average family size per household in the present study was 6-11; this is consistent with the findings of Asaminew and Eyassu, (2009) (family size of 8.2 and 7.2 in Bahir Dar and Mecha, respectively. On the other hand mean family size was reported 5.44 in the urban areas and 5.46 in the peri-urban areas (Tesfay, et al., 2012), which is lower, compared to the current study.

Cattle herd size

The present study indicated that crossbreed cows give more milk than the local cows; due to these households studied preferred to own large number of crossbreed cows. The households in the current study preferred having large number of female cattle to that of oxen in their total dairy herd. This is in line with Yoseph, et al., 2003. The proportion of crossbreed cows owned by a household is larger compared to that of the local ones. The households do this in order to increase the production of milk since milk production potential of the crossed cows is better than the local breeds. Yoseph, et al. (2003) reported that keeping more cows is also favored by urban and peri-urban dairy production system of Ethiopia who is targeting to produce more milk. In other reports by Kahsay, (2002), Workneh and Rowland, (2004) it was indicated that in pastoral management system also keeping more cows is practiced for the same purpose of producing more milk. This is indicative of the fact that keeping more cows is a common factor in cattle herding in many areas.

4.7.2 Dairy cattle husbandry practices

Feeds and feeding management

In the current study, majority of the farmers feed their animals with crop residue, natural pasture. Some give additional supplements including agro-industrial by-products and local brewery by-products. Asaminew and Eyassu, (2009) reported similar findings from Bahir Dar Zuria and Mecha Woredas. Furthermore, livestock feed resources in Ethiopia are mainly natural grazing lands and browses, crop residues, pasture, forage crop and agro-industrial by-products as reported by Alemayehu, (2005), Sintayehu, et al., (2008) in Shashemene area, Yitaye, (2008) Northwest Ethiopia and Kechero, et al., (2013) in Jimma Zone. On the other hand, Belete, (2006) reported that dairy producers using zero grazing were feeding their cattle by the cut-and carry system and are located around peri-urban and urban areas.

Water source and watering practices

In both zones of Arsi and East Showa the sources of water, which the farmers use, are river and tap water. The findings of the current study that showed that the majority was using tap water as sources of water, is similar to the result of Yitaye, 2008 which reported almost all of the farmers had access to pipe water for their livestock. However reports from Kedija, (2007) in Meiso district and Tesfaye, (2007) in Metema, Sintayehu, et al., (2008) in Dilla area, Kechero, et al., (2013) in Seka, Mana and Sedo indicated that river is the main source of water for livestock, following pipe water, lake and spring water. In addition reports from Belete, 2006 also differs from the present study. He reported that water from ground wells is the main sources of water following, rivers, lake, and tap water in Fogera District. This difference could be attributed to the level of difference in level of development that among the study areas.

The findings of the present study on frequency of watering dairy animals agreed with the reports of Lemma, et al., (2005) who reported that almost all the respondents watered their cattle twice in a day. On the other hand, our finding disagreed with Sintayehu, et al., (2008) in Dilla area, who reported 35.6% and 68% of the respondents watered their animals once a day in wet and dry season, respectively and also with Tesfaye, (2007) whose report stated that majority of households provide water for livestock once a day in wet season, where as in dry season they provide water twice daily.

Reproductive performance

Average age at first calving (28.97 ± 7.65 months) in the present study is shorter than the reports of Workneh and Rowland (2004) (47.61 months for agro-pastoral production system) Mulugeta, (2005) (44.5 months), Kedija, (2007) (52.49 months), Ayalew and Asefa, (2013) (47.16 months) in Ethiopia. In addition, the present result obtained is also shorter than the report by Kechero, et al. (2013) who reported 55.08 months in Seka, 57.72 months in Mana, 55.2 months in Dedo. However, the result obtained in the present study is similar with the reports of Getinet, et al. (2009) from Ogaden, (22.6 -51.5 months) and, Dolis, et al., (2010) (29.95 months). The difference could be due to factors like breed type, feeding and herd management in different Rehman, et al., (2008). In studies done by Abdel-Aziz, et al., (2005) and Ildikó, et al., (2006) breed was the most determinant factor in the case of age at first calving.

In the present study calving interval is 15.09 ± 4.63 months, which is in agreement with the findings of Workneh and Rowland, (2004) in pastoral area of Oromia region (15.5 months) and Gashaw (1992) in Selale (15.4 months). In addition, the present result is in line with the report by Kedija, (2007) who reported 16.01 months in Oromia region. However, calving interval in the present study was much lower than the calving interval found in North Shoa Zone by Ayalew and Asefa, (2013) (24.94 months for local cows). The same is true with Kechero, et al. (2013) which reported 24.86 months for Seka, 24.75 months for Mana and 25.47 months for Dedo. This difference could be attributed to the breed group and season of calving (Rafique, et al., 1999). In addition report from Rehman, et al., (2008) also indicated that feeding and reproductive management have significant effect on calving interval.

4.7.3 Milk production

Daily milk yield of 1.92 ± 0.88 liters by local breeds of dairy cattle in the present study is lower than report of Sintayehu, et al., (2008) and that of Yitaye, (2008) (2.8 liters per cow). Our finding is also lower than the one reported by Kedija, (2007) (2.37 and 4.80 liters in dry and wet season, respectively), and Belete (2006) 2 liters of the daily milk off take on the average from a local cow in peri urban and in urban areas). Daily milk yield of crossbred dairy cattle in the present study is 9.63± 4.47 liters which is quite similar to the reports of Belay, et al., (2012) (8.45±1.23 liters in Ethiopia) and also comparable with the findings of

Adebabay, (2009) (8 liters). However our finding is higher than the result reported by Yitaye, (2008) (7.8 ± 0.19 liters) and Belete, (2006) (7 liters in peri urban and in urban areas).

Milking and milk handling practices

Traditional hand milking is the major type of milking practices in the current study area; this is in agreement with the reports in Ethiopia by Kedija (2007), Bereda, et al., (2012) and (Tesfaye, 2007).

The report in Ethiopia (Kedija, 2007) which states that milking is usually under the control of women and there was no proper sanitary procedure followed during milking is partly in line with the present study. Similar studies in different parts of Ethiopia by Ayantu, (2006), Rahel, (2008), Derese, (2008), Sintayehu, et al., (2008), Kassahun, et al., (2011) and Bereda, et al., (2012) consent to the fact that dairying offers more opportunities for females to be closely involved in the daily management than other family members. These reports are in agreement with the findings of the present study. However, in some parts of Ethiopia there are few exceptions such as where milking is entirely performed by males as reported by Belete, (2006), Tesfaye, (2007) and Asaminew and Eyassu, (2009).

The present study found that majority of the farmers milked their cows twice a day and the time of milking is early morning and late evening. This finding agrees with the reports of Lemma, et al., (2005), Tesfaye, (2007), Yitaye, (2008), and Sintayehu, et al., (2008) in Dilla area, Kedija, (2007) and Yitaye, (2008) reported that cows were usually milked once or twice a day in Mieso district; however the report added that there are some farmers who milk their cows thrice a day. Ayantu's report, (2006) on milking frequency around Wolayta is in disagreement with the current study's result that milking was commonly done three times per day.

Contrary to the results of the current study, studies made by Fayo, (2004), Kedija, (2007), Derese, (2008), Bereda, et al., (2012) reported that majority of the respondents do not practice washing of teats during milking; the producers believe that during calf suckling for milk let-down, the teats get washed by the saliva of the calf and therefore, they think that it is not as such important to wash the teat before milking. On the other hand, Yitaye, (2008), Lemma, et al.

(2005), Haile, et al. (2012), Bereda, et al., (2012), Asaminew, and Eyassu, (2009) reported that the majority of the respondents practiced washing their hands and their milk utensils before milking, which is in consent with the present study. However, the cleaning is not efficient and the utensils are not properly dried; few of the farmers did not practice use of towel to clean the udder.

The present study shows that majority of the interviewed households used plastic jars and few used aluminium for milking. In agreement with the present study, Bereda, et al., (2012) reported houselolds used plastic jars as milking and transporting utensils. Similarly in urban farms, majority of the milk producers used plastic utensils and few used aluminums utensils. However, there were some urban farmers who used traditional clay pot utensils (Yitaye, 2008). On the contrary majority of the farmers in Metema district use traditional pot for milking and some were using small nickel (metallic) and plastic materials as reported by (Tesfaye, 2007).

Milk processing
As in the current study, a study conducted in Borena region confirms that Ayib (Ethiopian cottage cheese) and butter are the important products of smallholder dairy processing. The major milk producers in the present study area produced traditional butter (Kibe), Ethiopian cottage cheese (Ayib), traditional yoghurt (Ergo); some process milk into whey (Aguat). Similar studies by Ayenew, et al., (2009) in North western Ethiopia and Bereda, et al., (2013) in Gurage zone show that Ergo (Ethiopian naturally fermented milk), traditional butter (Kibe), traditional ghee (Neter Kibe), cottage cheese (Ayib), sour defatted milk (Arrera), and whey (Aguat) are the major milk products produced in their respective areas of study.

In the present study, milk processing at a household level is done by using traditional technology and the main products are butter (Kibe), sour milk (Ergo) and Ethiopian cottage cheese (Ayib) and this is in congruence with the study made by Teferee, (2003) and Yitaye, (2008). The majority of dairy producers use traditional churning material. In the case of the central highlands clay pot is mostly used (O'Mahoney and Peter, 1987). In East Wollega gourd is used for both churning and storage of milk (Alganesh, 2002).

4.7.4 Animal diseases and access to veterinary services

According to the report of Agajie, et al. (2002) in parts of Ethiopia animal diseases recognized by the farmers as the most serious, among others, are Tuberculosis (TB) Blackleg, Anthrax, Foot Mouth Disease (FMD) and this is very much in agreement with the current study. Kechero, et al., (2013), Tesfaye, (2007), Ayalew and Asefa, (2013) indicated that reproductive disorders and reproductive diseases, mastitis and gastrointestinal parasites are animal diseases encountered in different areas as it is the case in this study.

In accordance to the present study, record keeping is almost non-existent, which is in agreement with the reports of Sintayehu, et al., (2008). They reported that majority of the urban and mixed crop-livestock producers do not keep records. Record keeping in the current study area is not practiced most likely due to lack of adequate experience and/or awareness of the benefits of keeping records.

4.7.5 Main constraints and opportunities of the dairy sector

Dairy producers in the present study are constrained by different problems. They prioritized the major problems and constraints as high cost of feed and feed shortage; inadequate artificial insemination and veterinary services; and discouraging seasonal milk marketing. In addition, water scarcity, limitation of land for sustainable dairy development, high costs of inputs and lower prices of milk, and late payments for the milk sold were mentioned as limitations for the development of dairy production. Agreeing with the current study Agajie, et al., (2002), Belete, (2006), Asaminew and Eyassu, (2009), Sintayehu, et al., (2008), Ayalew and Asefa, 2013 reported that shortage and high costs of feeds, unavailability of artificial iInsemination (AI) and veterinary services, seasonality in demand for milk and milk products, access to farmland related problems, shortage of water supplies, high costs of inputs and lower prices of milk are the major constraints identified. They also reported disease prevalence and shortage of cash as the major constraints of dairy production.

Approximately 40% of the Ethiopian population is Orthodox Christians (Ahmed, et al., 2003). The calendar of the Orthodox Church involves long fasting periods per year and two fasting days every week (Wednesday and Friday), for a total of 180–250 days of fasting per year. During fasting days Orthodox Christians abstain from consuming products of animal origin,

including milk and dairy products (Ahmed, et al., 2003). During the long fasting times, the demand as well as the price of dairy products, particularly in the urban centers, where consumption of dairy products is relatively high, is highly affected. This is due to the low demand for dairy products during these days (Sintayehu, et al., 2008)

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CHAPTER 5

Identification of pathogenic bacterial species: *Escherichia coli*, *Listeria mocytogeneses*, *Staphylococcus aureus* and *Brucella* in raw bovine bulk milk in the selected milk collection centers of Arsi Dairy Union and Ada Dairy Cooperatives in Oromia Region Ethiopia

5. Identification of pathogenic bacterial species: *Escherichia coli, Listeria monocytogeneses, Staphylococcus aureus* and Brucella in raw bovine bulk milk in the selected milk collection centers of Arsi Dairy Union and Ada Dairy Cooperatives in Oromia Region Ethiopia

5.1 Abstract

The purpose of this study was to assess the microbial properties of milk produced by dairy farmers in Arsi and East Shewa Zones of Oromia Region in Ethiopia. A total of 106 samples of milk were collected; 50 samples from smallholder producers and 8 from Bel Deresa Dairy Farm in Arsi Zone and 48 from member smallholder producers in East Shoa Zone for microbiological invetsigation. The results showed that a total of 246 bacterial isolates were isolated from the 106 bulk milk samples. These included *Enterobacteriaceae* species (*E. coli)*, *Listeria* species (*Listeria monocytogenes*), *Staphylococcus* species (*Staphylococcus aureus*) and Brucella. Isolated *Escherichia coli* constituted 19.8%. In Lemlem Milk Collection Center (East Shewa Zone) *E. coli* was isolated from 80% of the samples whereas in Gobelecha Milk Collection Center in Arsi Zone the prevalence was low (15%). Out of *Listeria* species isolated 1.2% were *Listeria monocytogenes*: Denkaka (Debre Zeit) showed 10% prevalence, next to Kebele 02 (Debre Zeit) which showed 20% prevalence. *Staphylococcus aureus* constituted 3.2%: Lemlem and Babugaya milk collection center (East Shewa Zone) showed 20% prevalence. Brucella prevalence was 3.3%, Kebele 02 milk collection center (Debre Zeit) showed 40% prevalence and Kebele 11 milk collection center (Debre Zeit) and Bel Deresa dairy farm (Arsi Zone) showed 13% prevalence.

Keywords: Milk, smallholder producers, microbial qualities, *Escherichia coli, Staphylococcus aureus*, *Listeria monocytogenes* and Brucella, Ethiopia

5.2   Location, Materials and Methods

Study Location

Samples of milk were collected from two districts (Tiyo and Digeluna Tijo) and one dairy farm (Bel Deresa) in Arsi Zone and from Ada district in East Shewa Zone for microbiological analysis. They were all selected for the better and easy access they provided to quality milk.

5.3   Data Collection

On the basis of the information gained on hygienic practices during production and subsequent handling of milk from the first trial, an assessment of the bacteriological quality of bulk raw milk was conducted.

5.4   Sample size determination and sampling technique

Milk samples were collected from members of the Dairy Cooperatives for laboratory analysis as mentioned in the general methodology.

Milk sample collection

The approximate sample size required was estimated using the formula given by Arsham, (2005).

Milk sample allocation

Following proportional allocation, two milk collection centers (Assela town and Gobelencha) and one dairy farm (Del-Besira) were randomly selected in Arsi Zone and five milk collection stations (Kebele-11, Denkaka, Babugaya, Kebele-02, Lemlem) were randomly selected in East Shewa Zone. A total of 106 milk samples were proportionally allocated to the selected milk collection centers. Milk samples were randomly collected from each collection center.

Bacterial identification and isolation

Escherichia coli: One loop of milk was streaked on MacConkey agar plates, which is then incubated at 37^0C for 24hrs. Colonies that are pink colored were counted as presumptive *Escherichia coli.* Colonies picked from the MacConkey agar plates were re-streaked on fresh Eosin Methylene Blue (EMB) agar plates to purify and incubated at 37°C for 24hrs. Colonies that are green with metallic shine were counted as presumptive *Escherichia coli.* These colonies were transferred to Trypticase soy agar (TSA) plate for the following, Gram-stain,

oxidase test and catalase test. Gram negative, oxidase negative and catalase positive colonies were selected and used for identification of species. The identification was done using API 20 E biochemical test kit (BioMérieux, France).
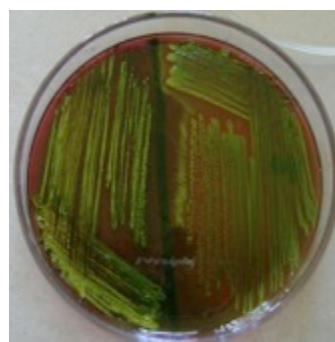
Figure X *Escherichia coli* on Maconkey agar, *Escherichia coli* on EMB agar,

Biochemical test result for *Escherichia coli*

Staphylococcus aureus : One loop of milk was streaked on Trypticase Soy Agar (TSA) plates, which are then incubated at 37^0C for 24 hrs. Those colonies that are white to golden yellow were counted as presumptive *Staphylococcus* species. Colonies picked from the Trypticase Soy Agar plates were re-streaked on fresh Trypticase Soy Agar plates to purify and then incubated at 37°C for 24 hrs. This was followed by Gram-stain, and catalase test. Gram-positive coccus colonies were selected and used for biochemical test. Catalase positive, Oxidation-Fermentation (OF) test positive and coagulase positive bacteria were confirmed as *Staphylococcus aureus* .
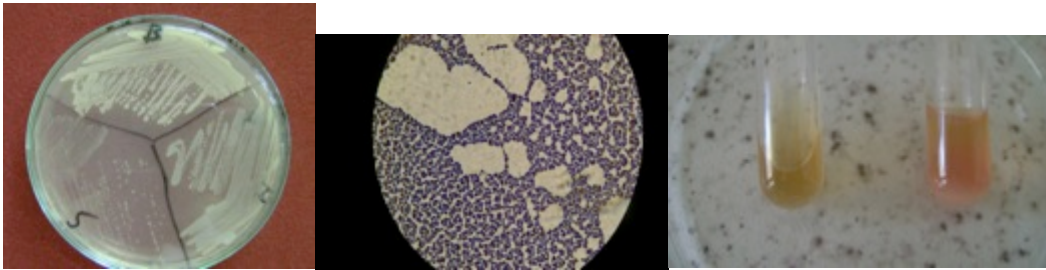
Figure XI Staph. On TSA agar, Staph. Microscope,   OF -ve and OF +ve

Listeria monocytogenes: 25ml of milk sample was homogenized with 100ml of Half Fraser broth and incubated at 30ºC for 24 hrs. After 24hrs the sample was transferred using a loop and streak over the surface of Rapid *Listeria monocytogenes* (RLM) agar plate and incubated at 37ºC for 48 hrs for further isolation. Colonies that are gray-blue, round and smooth precipitate were counted as *Listeria monocytogenes*. Colonies of *Listeria monocytogenes* were selected and used for species identification using Microgen *Listeria* ID.

Figure XII   *Listeria, Listeria m.* on RLM,   Biochemical test

Brucella - Milk ring test (MRT): 1 ml of milk sample was put in a test tube (25mm height) and 3µl of the standard MRT antigen (killed *B. abortus* stained with hematoxylin) was added then mixed well. The mixture was left for an hour at 37ºC. Positive samples were identified as blue cream layer over distained milk while negative samples were identified as whitish cream layered over stained milk.

The MRT works on the principle that antibodies to *B. abortus* attach themselves to fat globule agglutinins in milk that rise to the surface of the milk and cluster in the cream layer. When haematoxylin stained *B. abortus* antigen combines with Brucella antibody, a complex, which adheres to the fat globules in the cream layer of milk, is formed.
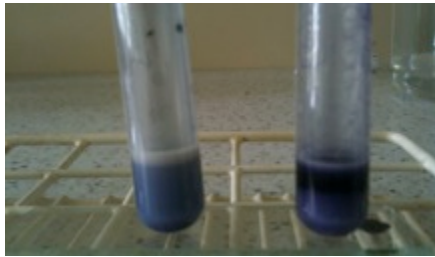
Figure XIII   MRT negative and MRT positive

5.5   Data analysis

All the collected data were stored and managed in Microsoft Excel database. The data were analyzed using the statistical software SPSS (Version 16.0, 2006). The results for microbial statistical in different primary diary cooperatives were obtained using descriptive statistics. Significant differences were considered as ($p<0.05$).

5.6   Results

5.6.1 Bacterial pathogens isolation and identification

106 bulk milk samples, collected from dairy farms in the two zones, were analyzed for bacterial isolation. Among the bulk milk samples analyzed a total of 247 isolates were obtained including *Enterobacteriaceae, Listeria*, *Staphylococcus* and Brucella antibodies. *Enterobacteriaceae* species constituted 37.2% of the isolates and *Escherichia coli (E. coli)* represented 19.8% of the *Enterobacteriaceae* species, *Staphylococcus* accounted 16.6%, and out of this 3.2% were *Staphylococcus aureus.* 42.9% of the isolates were *Listeria* species out of which 1.2% was *Listeria monocytogenes.* The prevalence of Brucella was 3.3% (Table V).

Table V Pathogenic Bacteria isolates from 106 bulk milk samples from Asella Dairy Union and Ada Primary Dairy Cooperative, Ethiopia (2010)

Type of bacteria	No of bacteria isolated	Percent prevalence
Enterobacteriaceae	92	**37.2**
E. coli	49	**19.8**
Other Entro	43	**17.4**
Listeria	106	**42.9**
Listeria monocy.	3	**1.2**
Other Listeria	103	**41.7**
Staphylococcus	41	**16.6**
S. aureus	8	**3.2**
Non patho Staph	33	**13.4**
Brucella	8	**3.3**
Total	**247**	

5.6.2 Bacterial pathogens isolates in the different PDCs

Prevalence of *Escherichia coli* isolates is much higher in Lemlem milk collection center in East Shewa Zone than the other milk collection centers. Lemlem milk collection center showed 80% prevalence followed by Kebele 02 (70%) and Denkaka (60%) whereas lower isolates were found in Gobelench (15%) in Arsi Zone.

Figure XIV Pathogenic Enterobacteriaceae isolates in the different PDCs from Asella Dairy Union and Ada Primary Dairy Cooperative, Ethiopia (2010)
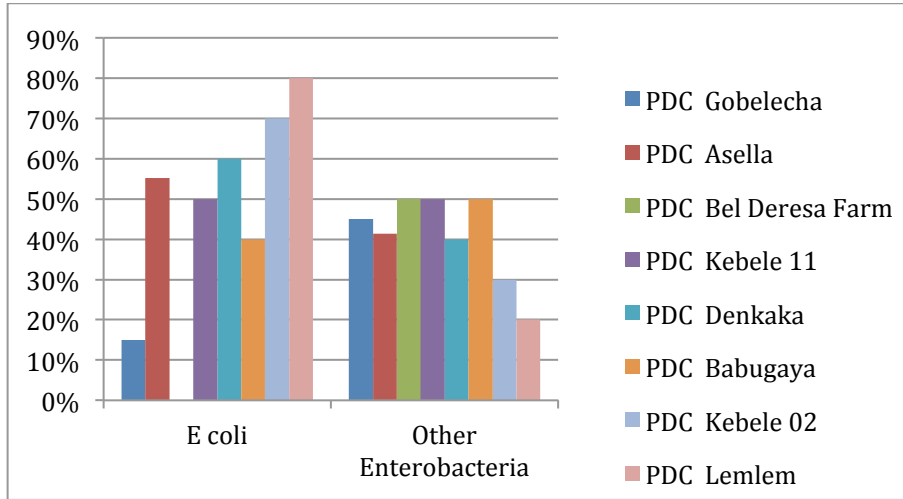
Higher prevalence of pathogenic *Listeria monocytogenes* was detected in Kebele 02 milk collection center (20%). This is followed by Denkaka milk collection center (10%) in East Shewa Zone. There was no prevalence of *Listeria monocytogenes* detected in the other collection centers.

Figure XV Pathogenic *Listeria* isolates in the different PDC from Asella Dairy Union and Ada Primary Dairy Cooperative, Ethiopia (2010)
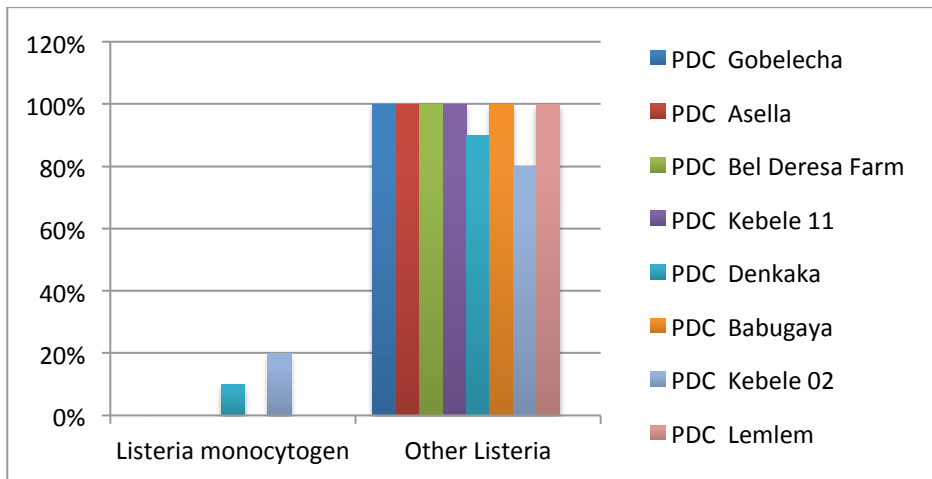
Higher prevalence (20%) of *Staphylococcus aureus* was detected in Babugaya and Lemlem milk collection center in East Shewa Zone. Next higher prevalences

were found in Gobelencha (15%), Arsi Zone and Kebele 02 (10%), East Shewa Zone while in the rest collection centers there was no prevalence detected.

Figure XVI Pathogenic *Staphylococcus* isolates in the different PDC from Asella Dairy Union and Ada Primary Dairy Cooperative, Ethiopia (2010)

Higher prevalence of Brucella (40%) was found in Kebele 02 milk collection center in East Shewa Zone. Kebele 11 milk collection center and Bel-Deresa Farm showed 13% prevalence. In Arsi Zone only Asella milk collection center showed Brucella prevalence, which is 3%.

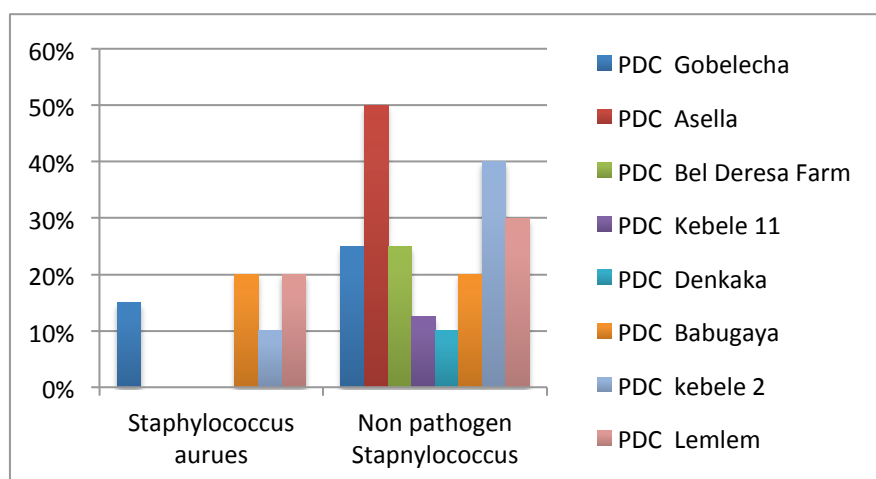Figure XVII Pathogenic Brucella in the different PDC from Asella Dairy Union and Ada Primary Dairy Cooperative, Ethiopia (2010)
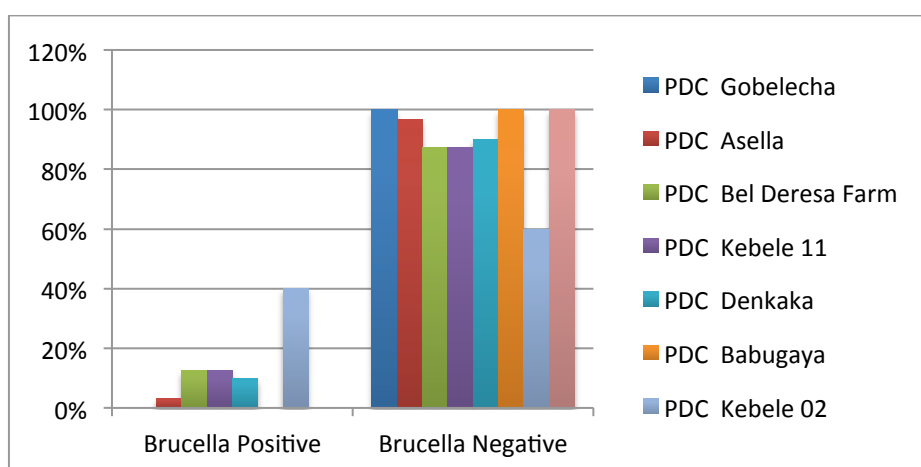
5.7 Discussions

5.7.1 Staphylococcus aureus

Staphylococcus aureus is a common cause of community and hospital acquired infections. It is associated with infections in all age groups, including surgical wounds, skin abscess, osteomylitis, septicemia, food poisoning and toxic shock syndrome (Jawetz, et al., 2007). The prevalence of *Staphylococcus* identified in the present study was (16.6%). This result was lower when compared to the results found by Girma, (2001) (42.5% in Ethiopia), Workineh, et al., (2002) (57% in Ethiopia), as well as Kerro and Tareke, (2003) (39.2%), Matios, (2009) (41.4%,) and Bedane et al., (2012) in Ethiopia (29.2%).

In the present study, the prevalence of *Staphylococcus aureus* was 3.2% in bulk tank milk. The 2.2% contamination rate by *Staphylococcus aureus* reported in the United Kingdom (Bradley and Green, 2001) is quite similar to the contamination rate found in the current study. Higher results to the current study were also found. Likewise Mekonnen, et al., (2011) reported 8% of the bucket milk and 10% tanks milk samples were contaminated with *Staphylococcus aureus*. Other studies also found higher contaminations for *Staphylococcus aureus*, which are comparable with the findings referred above. According to the study made by Normanno, et al., (2007), 17% contamination from milk and dairy samples was found. In Bahir Dar town, in Amhara Region, according to Betaw, et al., (2010) higher prevalence contamination of (20.3%) in dairy farms was reported. Quite higher results in other place in Ethiopia, were also reported, 40.5% in Repi and Debre Zeit dairy farms (Workineh, et al., 2002) and 40.6% reported by (Daka, et al., 2012) in Hawassa.

Apart from studies in Ethiopia, several higher contamination results were reported. Forough, et al., (2012) reported higher results of 17.9% in Iran. (Gianneechini, et al., 2002) reported (37.5%) from clinical cases of mastitis in Uruguay. There are also other higher results reported from South Korea and Morocco. They are prevalence of 29.6% by (Lee, 2003) and 40% by Bendahou, et al., (2008) respectively. Contamination rate of 8.3% in India reported by Shah, et al., (1985). In Brazil by Fagundes, et al., (2010) also prevalence of 10.8% was found from bulk tank milk. Thaker, et al., (2013) also found higher contamination of 6.25 % in India.

5.7.2 Escherichia coli

Escherichia coli (*E. coli*) is capable of causing widespread infectious diseases like intestinal and extra intestinal types (Santo, et al., 2006). In this study, the prevalence of *Escherichia coli* was 19.8% in bulk tank milk sample. 12.91% of contamination in Ethiopia was reported by Worku, et al., (2012), which is comparable to the present finding. Comparable results of prevalence from other countries were also reported. Gianneechini, et al. (2002) found *Escherichia coli* of isolation rate of 12.5% from clinical cases of mastits in Uruguay and Akabanda, (2010) in Ghana found 20% of contamination of samples with *Escherichia coli*.

Asamenew, et al., (2012) found 11.4% contamination rate with *Escherichia coli* in Ethiopia and Ayano, et al., (2013) reported 11.6% prevalence of *Escherichia coli* in Ethiopia. However, higher contamination in comparison with the current study was also found. Van Kessel, et al., (2004) found *Escherichia coli* prevalence of 93% from milk samples in US. A number of other studies reported higher prevalence of *E. coli* (Girma, 2001) (39.2%), Haimanot, et al., (2010) (26.6%) and Katsande, et al., (2013) (25.2%)

5.7.3 Listeria monocytogenes

Listeria species are found worldwide and everywhere in animals, foods, humans, soil, the food-processing environment, and contact surfaces and also in food containers. Of the several species of *Listeria*, *Listeria monocytogenes* is an important cause of wide spectrum of diseases in animals and humans (Todar's, 2003). Out of the milk samples tested in the present study, a total of 42.9% were found to be *Listeria* contaminated. Quite similar to the present study, contamination a 41.4% incidence of *Listeria* was reported by Akman, et al., (2003). According to Molla, et al., (2004) from food samples examined in Ethiopia, 32.6% of the samples were found to be positive for *Listeria*.

There are also lower prevalence reported by some authors. In Malaysia, 4.6% contamination of milk samples with *Listeria* was found (Chye, et al., 2004). According to Rahimi, et al. (2012), an overall prevalence of 7.2% was reported in Iran. In Uganda, 60% of the samples tested positive for *Listeria* in as reported by Mugampoza, et al., (2011). This value is higher the result obtained in the present study. The prevalence of *Listeria monocytogenes* was 1.2% in the present study, which is in agreement with a previous study made in Sweden (1.0%) from bulk

tank milk as reported by Waak et al., (2002). The finding of the present study (1.2%) is also in congruence with the prevalence of 1.9% in Malaysia as reported by Chye, et al., (2004). Furthermore, Navratilova, et al., (2004) found prevalence of 2.1% from raw milk in Czech Republic; Tasci, et al., (2010) reported a prevalence of 1.17% from raw milk in Turkey. However, contamination of milk samples by *Listeria monocytogenes* reported by Van Kessel, et al., (2004) (6.5%) in the US and Molla, et al., (2004) (5.1%) from food samples in Ethiopia were quite higher than the current finding. Moreover, Vilar, et al., (2007) reported higher prevalence (6.1%) from bulk milk samples in Spain and Frehiwot (2007) reported 5.4% prevalence from samples taken from dairy products in Ethiopia.

5.7.4 Brucella

Brucellosis remains widespread in the livestock populations, and represents a great economic and public health problem in African countries. Brucellosis causes abortion, which is the major means of spread by infected afterbirth or fetus as well as excretion of excessive organisms which can easily be acquired by susceptible animals (McDermott and Arimi, 2002). Overall prevalence of Brucella using milk ring test (MRT) in the present study is 3.3% from bulk milk samples. Similarly to the present study milk samples collected during spring (1.22%) and autumn (1.17%) seasons showed positive reaction with milk ring test obtained in Iran (Maadi et al., 2011). Other finding, prevalence of brucellosis found at consumer-level as determined by MRT was 3.9% and at the informal market level was 3.4% in Kenya as reported by Kang'ethe et al., (2000). In Nigeria, Farouk, et al., (2013) also revealed similar result to the present study from fresh milk samples (3.4%) tested positive for MRT.

Compared to the present study, higher results of Brucella antibodies were obtained. In the study made by Islam, et al in Bangladesh, (2010) 13.64% of the milk samples recorded positive by MRT Brucella. Brucella was detected in 21.9% of the milk samples studied, using MRT, by (Mensah, et al., 2011). Prevalence of 18.61 % of the milk samples were positive according to the MRT reported by Cadmus, et al., (2008); it was reported that Terzi, (2006) found prevalence of 20% Brucella antibodies from cow milk in Turkey. Prevalence rate of bovine brucellosis when using milk ring test, was 32.5% in Khartoum State (Salman and El-Nasri, 2012). In addition 7.1% prevalence of Brucella positive cattle milk sample reported in Pakistan by Saleha, et al., (2014). The data collected by

Shafee, et al., (2011) revealed prevalence (8.5%) from bovine milk samples using MRT in Pakistan. Another finding from pooled milk samples from milk markets in Nigeria (7.4%) tested positive to the Milk Ring Test according to Farouk, et al., (2013). Finding made on camel milk samples from Kenya were tested using the Milk Ring Test (MRT) and out of the total samples (15.36%) tested positive Wanjohi, et al., (2012).

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CHAPTER 6

Occurrence of Methicillin Resistant Staphylococcus aureus and Enterotoxigenic Escherichia coli (O157) in raw bovine bulk milk in the selected milk collection centers of Arsi Dairy Union and Ada Dairy Cooperatives in Oromia Region Ethiopia

6. Occurrence of Methicillin Resistant *Staphylococcus aureus* and Enterotoxigenic *Escherichia coli* (O157) in raw bovine bulk milk in the selected milk collection centers of Arsi Dairy Union and Ada Dairy Cooperatives in Oromia Region Ethiopia

6.1 Abstract

This study was conducted to assess hygienic practices during production and the microbial properties of milk in Arsi Zone in Oromia, Ethiopia. A total of 100 samples of milk were collected and analyzed. Among the total milk samples analyzed for each pathogen, 50.5% were *Staphylococcus* of which *Staphylococcus aureus* were (5.05%), and (47.5%) *Escherichia coli* were also analyzed from the (49.5%) Enterobacteriaceae isolated. Out of the *Staphylococcus aureus* recovered, all the isolates were sensitive to Methicillin Resistant *Staphylococcus aureus* (MRSA) test and 27.8% of the recovered *Escherichia coli* was positive for E coli O157 serotype. Out of the total isolated *Escherichia coli 39*% was in Tiyo district, 35% in Lemuna Bilbilo district and the 20% was in Digeluna Tiyo district. With regards to the serotype test E coli O157 recovered 22% was in Tiyo district whereas 18% was in Lemuna Bilbilo district and 15% in Digeluna Tiyo. *Staphylococcus aureus* in Tiyo district was 8% and the one in Lemuna Bilbilo district was 2%. All of *Staphylococcus aureus* recovered in each district was sensitive to Methicillin Resistant *Staphylococcus aureus* test. The differences in isolated *Escherichia coli* O157 across the districts were not statistically significant ($p<0.05$). On the other hand sensitivity for Methicillin Resistant *Staphylococcus aureus* in each district was statistically significant ($p<0.05$). In the different PDCs, the total isolated *Escherichia coli* was 15% in Bilalo, 24% in Assela town, 17% in Lemmu Ariya, 18% in Bekoji and 20% in Gobelencha. Whereas the serotype E coli O157 recovered in Bilalo was 9%, in Assela town it was 13%, in Lemmu Ariya 8%, in Bekoji 10% and 15% in Gobelencha. While *Staphylococcus aureus* in Bilalo was 3%, Assela town 5%, Lemmu Ariya 1%, Bekoji 1%, there was no isolates in Gobelencha. According to the *Staphylococcus aureus* test in each PDC, all of the

Staphylococcus aureus recovered was sensitive for Methicillin Resistance test. The differences in isolated *Escherichia coli* O157 across the PDCs were not statistically significant (p<0.05). On the other hand sensitive for Methicillin Resistant *Staphylococcus aureus* test in each PDC was statistically significant (p<0.05).

Keywords: Milk, microbial qualities, *Escherichia coli* O157, Methicillin Resistant *Staphylococcus aureus* (MRSA), Ethiopia

6.2   Location, Materials And Methods
Study Location
Samples of milk were collected from three districts namely, (Tiyo, Digeluna Tijo, and Lemuna Bilbilo) in Arsi Zone.

6.3   Data Collection
Based on the bacteriological test made earlier, two important bacteria were picked for further study, which are *Escherichia coli* for Serotype O157 and *Saphylococcus aureus* for Methicillin Resistant *Saphylococcus aureus.*

Milk samples were collected from members of dairy producing farmers in the cooperatives for laboratory analysis as mentioned on chapter 5.

6.4   Sample size determination and sampling technique
Milk sample allocation
Following proportional allocation, five milk collection centers (Asella town, Bilalo, Lemu Ariya, Bekoji, and Gobelencha) from Arsi Zone were selected. Total of 100 milk samples were proportionally allocated to the selected milk collection centers. Milk samples were randomly collected from each collection center.

Bacterial identification and isolation
Escherichia coli **Serotype O157**: identification was made using Dry spot Latex test.

Dry spot Latex test: a drop of the test latex was dispensed onto a circle of the reaction card placing it close to the edge of the circle. A drop of saline solution (9gr/L NaCl) was added to the circle. Using a loop, a portion of the colony is

picked to be tested and emulsify in the saline drop till it gets smooth. The test latex was mixed together and spread to cover the reaction area using the loop. Rocking was made to the card in a circular motion for a minute for observing agglutination. Agglutination of the test latex within a minute was interpreted as positive for the presence of *Escherichia coli* Serogroup O157 and no agglutination within a minute is interpreted as negative for the presence of *Escherichia coli* Serogroup O157.
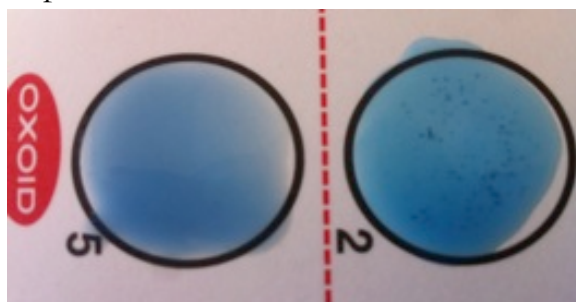
Figure XVIII   No Agglutination (negative) and Agglutination (positive)

Methicillin Resistant *Saphylococcus aureus*: identification was made using Kirby-Bauer Method.

Kirby-Bauer Method: uses Mueller-Hilton agar and used to test Methicillin Resistant *Saphylococcus aureus*. 2-3 colonies were suspended into 5ml of saline solution (9gr/L NaCl). Using a cotton swab the suspension was plated on the Mueller-Hilton plate and seeded through the plate then put the disk on the agar and incubated at 37^0C for 24 hrs. The diameter of the inhibition was measured to interpret the result. Interpretation was made as Resistant for Methicillin when the diameter is ≤ 9mm, Intermediate for diameter between 10-13mm and Sensitive for Methicillin with the diameter ≥ 14mm.

Figure XIX   Negative for sensitivity test and Positive for sensitivity test

6.5 Data analysis

All the collected data were stored and managed in Microsoft Excel database. The data were analyzed using the statistical software SPSS (Version 16.0, 2006). The data in the different districts and primary diary cooperatives were summarized using descriptive writing. Significant differences were considered as (p<0.05).
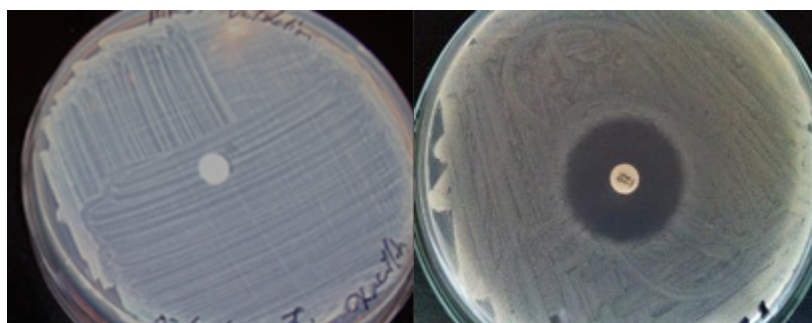
6.6   Results

6.6.1   Bacterial pathogens isolation and identification

100 bulk milk samples collected from Arsi zone were analyzed for bacterial isolation. Among the bulk milk samples analyzed a total of 198 isolates were obtained including pathogenic Methicillin Resistant *Staphylococcus aureus* (MRSA) and Enterotoxigenic *Escherichia coli* (ETEC) (O157) in raw bovine milk in the selected milk sheds of Asella Dairy Union.

Among the total milk samples analyzed for each pathogen, 50.5% were *Staphylococcus* of which *Staphylococcus aureus* were (5%), and out the (49.5%) Enterobacteriaceae isolated, *Escherichia coli* were (47.5%). Out of the *Staphylococcus aureus* recovered all the isolates were sensitive to Methicillin Resistant *Staphylococcus aureus* (MRSA) test and 27.8% of the recovered *Escherichia coli* was *E coli* O157 serotype (Table 7)

Table VI Pathogenic Bacteria isolates

Type of bacteria	No. of bacteria isolated	Percent prevalence
Enterobacteriaceae	98	**49.5**
Escherichia coli	94	**47.5**
Other Entro.	4	**2.0**
Staphylococcus	100	**50.5**
Staphylococcus aureus	10	**5.05**
Non-patho. *Staph*	90	**45.5**
Sensitive to MRSA test	10	**5.1**
Serotype *Escherichia coli* O157	55	**27.8**
Total	**198**	

6.6.2   Bacterial pathogens isolates in the different Districts

Out of the total isolated *Escherichia coli (E. coli)* 39% was in Tiyo district, 35% in Lemuna Bilbilo district and 20% was in Digeluna Tiyo. In Tiyo district, the serotype *E coli* O157 recovered was 22% whereas it was 18% in Lemuna Bilbilo district and 15% in Digeluna Tiyo. *Staphylococcus aureus* in Tiyo district was 8% and 2% in Lemuna Bilbilo district. All of *Staphylococcus aureus* recovered in each district was sensitive to Methicillin Resistant *Staphylococcus aureus* test. The differences in isolated *Escherichia coli* O157 across the districts were P=0.089 which is not statistically significant (p<0.05). On the other hand sensitivity for Methicillin Resistant *Staphylococcus aureus* test in each district showed P=0.020 which is statistically significant (p<0.05).

Figure XX Bacterial pathogens isolates in the different Districts
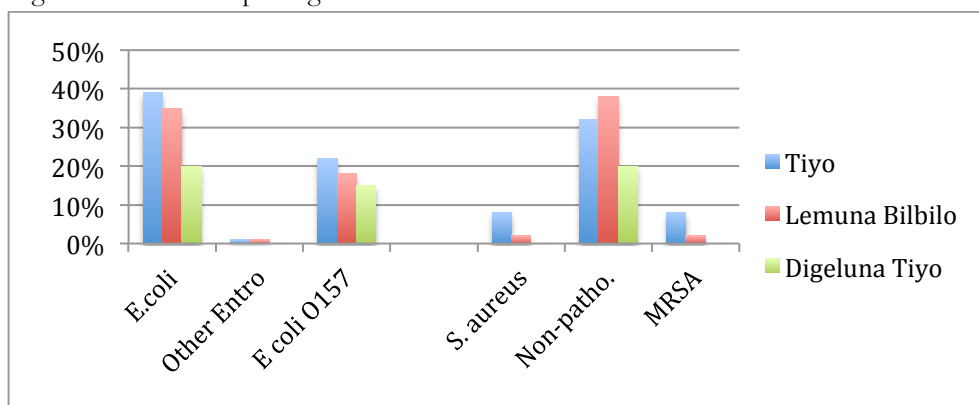
6.6.3   Bacterial pathogens isolates in the different PDCs

In the different PDCs, the total isolated *Escherichia coli (E. coli)* varied in percentage; in Bilalo it was 15%, in Assela town 24%, Lemmu Ariya 17%, Bekoji 18% and 20% in Gobelencha. The serotype *E coli* O157 recovered in Bilalo was 9%, in Assela town it was 13%, Lemmu Ariya 8%, Bekoji 10% and 15% in Gobelencha. *Staphylococcus aureus* in Bilalo was 3%, Assela town 5%, Lemmu Ariya 1%, Bekoji 1% and in Gobelencha there was no isolates. All of *Staphylococcus aureus* recovered was sensitive for Methicillin Resistant *Staphylococcus aureus* test in each PDC. The differences in isolated *Escherichia coli* O157 across the PDCs were P=0.261 which is not statistically significant (p<0.05). On the other hand sensitivity for Methicillin Resistant *Staphylococcus aureus* test in each PDC showed P=0.098 which is statistically significant (p<0.05)
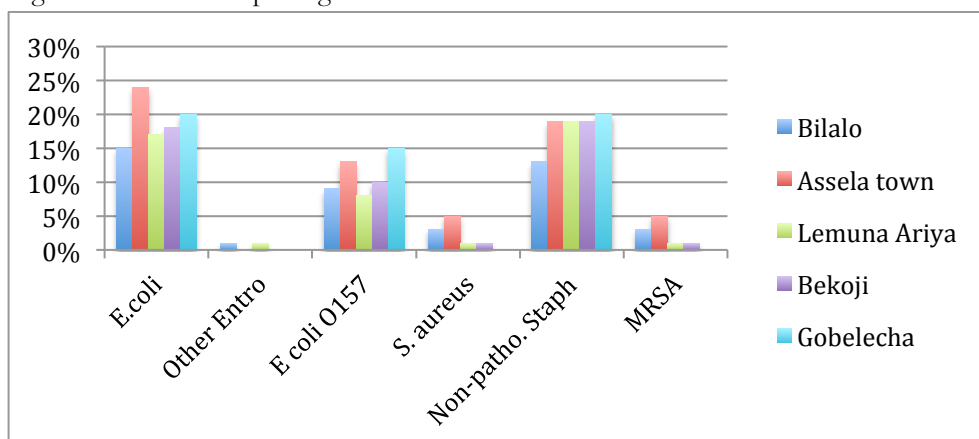
Figure XXI Bacterial pathogens isolates in the different PDCs

6.7 Discussions

6.7.1 Methicillin-resistant Staphylococcus aureus (MRSA)

The increasing frequency of antimicrobial resistance among infectious organisms is of great concern to both medical providers and the general public (Herold, et al., 1998). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare associated infections worldwide and has recently been identified as an emerging pathogen in livestock and companion animals. The samples taken and identified in the present study are found to be sensitive for the MRSA test; it is in line with the findings by Virgin, et al., (2009) in US, which showed that bulk tank milk tested positive for *Staphylococcus aureus.* However, none were positive for Methicillin-resistant *Staphylococcus aureus on* the selective indicator medium. Huber, et al., (2010) in Switzerland tested food samples such as BTM, raw milk cheese for Methicillin-resistant *Staphylococcus aureus and* found that there were no positive results. Zinke, et al., (2012) in Germany also reported cheese samples were positive for *Staphylococcus aureus;* but Methicillin-resistant *Staphylococcus aureus was* not detectable in any of the cheese samples. While other studies found a slight difference with the findings referred to above, MRSA carriage rate was found to be 1% on dairy cows in the study made by Stien, et al., (2008). Olde Riekerink, et al., (2008) reported 2% herd prevalence in Dutch. According to Haran, et al., (2012) 4% isolates were recovered from Bulk Tank Milk in Minnesota.

6.7.2 Escherichia coli O157

Escherichia coli (E. coli) are normal part of the intestinal micro-flora of many healthy animals, including humans. However, some strains can cause diseases

including serotype O157:H7, which are among groups that are capable of causing severe, chronic, and potentially fatal illness (Tarr, 1995). Overall *Escherichia coli* O157 serotype identified in the present study was (27.8%) which, is quiet lower than the following findings; (34.6%) of samples positive for *Escherichia coli* O157 from dairy animals in Malaysia (Chapman, et al., 1997), prevalence of 33.5% for *Escherichia coli O157:H7* isolated from raw milk samples by Chye, et al., (2004), and the overall farm prevalence for *Escherichia coli* O157 which was 37.8%. The findings reported by Cobbaut, et al., (2009) indicating the prevalence for *Escherichia coli* O157 in Belgium dairy cattle farms as 61.2% was found to be quiet high. However, the prevalence of *Escherichia coli* O157 (22.7%) found on beef farm in Belgium (Cobbaut, et al., 2009) is very close to the current finding, which is 27.8%. According to the studies by (Samadpour, et al., 1994) found that 17% samples in different food (Fresh meat, poultry, and seafood) in US, (Chapman, et al., 1997) found 15.7% in cattle, (Mora, et al., 2007) found 12.3% in food samples (beef, soft cheese, fresh vegetables) and (Lye, et al., 2013) found 18.75% in raw cow milk in Peru were positive for *Escherichia coli* O157. These studies compared to the finding of the present study are found to be lower results. Observation of the Tiyo district showed higher prevalence (22%). Reports by (Mora, et al., 2007) in Lima, Peru on prevalence in ground beef which is 22.5% as well as the one reported by Cobbaut, et al., (2009) in Belgium which is 22.7% is similar with the one observed in Tiyo district (22%). The findings in raw cow milk (18.75%) shown by study made by Lye, et al., (2013) and 17% prevalence in different food reported by (Samadpour, et al., 1994) was similar to the result in Lemuna Bilbilo district. The low prevalence found in Digeluna Tiyo district, 15% is in line with 12% prevalence of *Escherichia coli in* Irish dairy cattle (Murphy, et al., 2005).

6.8 Reference

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CHAPTER 7

General Discussion

7. General Discussion

Milk and milk products are highly perishable foods and unless correctly processed, packaged, distributed and stored they may become potentially unsafe due to growth of pathogenic microorganisms. The key factor to ensure good quality milk production and food safety is to avoid contaminations (Elmoslemany, et al., 2009). To ensure food safety and public health it needs critical involvement of feed hygiene, hygienic dairy farm practice and dairy processing (Valeeva, et al., 2005). Failure to maintain adequate sanitation and hygienic practices will contribute to the contamination of milk by undesirable or pathogenic microorganisms.

The present study shows that faulty handling, in some dairy farmers milking procedures, where there is no proper cleaning of milking utensils or properly washing hands immediately before milking can lead to the contamination of the milk. The current study further indicates that even those respondents who practiced washing their hands before milking, clean the milking utensils as well as the teats of the cows may not be much of guarantee for the safety of the milk. This is due to several factors. The cleaning is not properly done, so it is not effective; in addition, the so-called cleaned utensils are not properly dried up to ensure safety. On top of this, it was clear that for few of the respondents, the cleanliness of the sources of their water is far from certain. They get their water from rivers which in most cases are not clean; the use of this water for cleaning hands, udder and milking utensils prior to milking cannot guarantee avoiding or preventing contamination of the milk. These are possible contributing factors for the high contamination of milk during milking in the current study area and for other problems ensuing from this.

Galton, (1986) and Gonfaa, et al., (2001) also noted that insufficiently cleaning the udder before milking and lack of other pre-milking hygienic preparations plays an important role in the contamination of milk during milking. In Malaysia possible reasons for the high bacteria counts discovered in the milk were found to be due to unhygienic milking procedures or equipment and poor quality of water used for cleaning utensils and animals. In addition, utensils were not often washed and dried properly and their walls are subject to great risk of contamination growth (Chye, et al., 2004) and (Millogo, et al., 2010). Also, the number of personnel working at milk collection centers during the time of milk

collection was higher which might have contributed to milk contamination (Mekonnen, et al., 2011). Given the deficiency of keeping ones clothes free from problems related to bacterial contamination due to lack of clean water and the inability to provide clean gowns to the workers, having more personnel working in the milk collection area adds to the problem of contaminating the milk.

Microbial counts in raw milk depend not only on contamination during milking but also on the temperature at which milk is stored and on the time that elapses between milk production and collection (Soler, et al., 1995) and (Ahmed, et al., 2010). This very well defines high bacterial contamination in our study, which is due to long duration of milk transport to the cooperative center. According to Garedew, et al., (2012), the detection of bacterial contamination also might be attributed to higher environmental contamination during transportation and/or contamination during waiting along the roadside. Also, (Farah, et al., 2007) added that the conditions of roads and the distance travelled by the producers creates conducive environment for multiplications of bacteria and milk contamination and decrease the milk quality up to spoilage. In many cases, farmers in our study area dispense their milk in two ways. When they want to sell the milk fresh they transport it to the nearby milk cooperatives; if the cooperatives cannot take the whole fresh milk directly, the farmers will process it into more shelf stable product.

During transporting the milk to the cooperative centers, there is no chance of cooling the milk, as there is no cooling system in place; the first cooling of the milk is done at the milk cooperative centers. Major transport means of milk in current study area is by foot, horse/donkey, bicycle and public transport. The time that elapse between the milking time and the cooling of the milk has huge influence for the milk to be contaminated. The finding by (Chye, et al., 2004) indicated that milk contamination depends on milk storage conditions; it further stated that milk storage condition is also basic determinant for milk quality. This finding is very much in line with and justifies the findings of the current study. Other supporting finding to this is the one by Bonfoh, et al., (2003) and Millogo, et al., (2008). It states that the lack of facilities such as electricity and cooling systems are additional reasons for increased milk contamination during transport and storage of milk. The finding of Srairi, et al., (2006) is yet another finding which is in support of the current study's finding; it states that lack of milk

refrigeration in the situation of long distance milk transportation is the potential source of infection.

Omore, et al., (2003) mentioned that bacterial contamination increases with bulking and number of agents handling milk during collection. Garedew, et al., (2012) as well reported that bacterial isolates from bulk tank milk at milk collection center might result from unnecessary mixing, transfer of milk from can to can and long milk collection rounds coupled with high ambient temperature. Milk is generally exposed to different contaminants when it is transferred from one container to another, transported to collection centers as well as from the production site to processing plants without cooling facilities, and with no proper milk containers (Welearegay, 2012).

Utensils used for milking and milk transport also contribute to the poor hygienic quality of the raw milk. The bacteriological result identified in our study is very much related to the type and cleanliness of the milk utensils used for milking and milk transportation. Majority of the farmers in the present study used plastic utensils, some of which are designed with small openings, and few of the farmers used traditional pots that are difficult to clean. According to Omore, et al., (2005) plastic containers were associated with high coliform counts in raw milk and Donkor, et al., (2007) added that coliform bacteria can rapidly build up in moist milky residues in plastic containers; the design of some restricts access to the bottom of the container. Additional findings, by (Affif, et al., 2007; Rysanek, et al., 2007; Grimaud, et al., 2007; Millogo, et al., 2010) indicated that   the various utensils like plastic buckets and bulk storage tanks used during milk collection usually constitute the source of greatest contamination of milk. The initial microbiological quality of the milk varies considerably and depends for the most part on the cleanliness and types of containers as Mwangi, et al., (2000); Parekh and Subhash, (2008) revealed in their findings. As reported by Bonfoh, et al., (2003) in Mali, the traditional wooden calabash milk containers and most of the plastic containers are porous and make cleaning difficult.

The prevalence of foodborne pathogens in milk is influenced by numerous factors such as farm size, number of animals on the farm, milking hygiene, farm management practices, and variation in sampling, types of samples evaluated, geographical location and season. All of the bacteria identified from the bulk milk samples collected directly from the collection centers in the current study

were positive for coliform bacteria *Escherichia coli*, *Escherichia coli* O157, *Staphylococcus aureus* , *Listeria monocytogenes* and Brucella; but negative or sensitive result was found for MRSA.

As stated by (Bonfoh, et al., 2003), the detection of coliform bacteria or pathogens in milk causing udder infection is due to contaminated milking utensils and water supply. According to Maity, et al., (2010) and Sim Kheng Yuen, et al., (2012), contamination of milk and milk products with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions.

Escherichia coli is one of the bacteria that exist in the normal micro flora of the intestinal tract of humans and warm-blooded animals. In the current study we found higher *Escherichia coli* contamination; this reveals that there is lack of hygienic milking, handling, storage, transportation and that there is also fecal contamination of milk. Our justification for the current finding is very much in line with other researches made. Soomro, et al., (2002) indicated that *Escherichia coli* is reliable indicator of fecal pollution, in general, in water, food and milk and other dairy products. Hassan, et al., (2012) also identified that using contaminated or poor quality water for personal hygiene, cleaning utensils and animals can be another means of milk contamination with *Escherichia coli.* Our study found few farmers use river water for personal hygiene, cleaning utensils and animals, which is supported by Hassan, et al., (2012). According to the observation of Haimanot, et al., (2010), Parekh and Subhash, (2008) and Meshref, (2013), the detection of *Escherichia coli* in milk can be caused by unhygienic food processing practice and fecal contamination.

Smallholder farmers in the present study practice traditional ways of milk production, a situation where use of disinfectants and gloves are not exercised. Lack of technology and on top of this not using disinfectants and clean disposable gloves during milking and other milk production exercise, exposes the milk to various bacterial attack; and this can be an additional cause for the occurrence of high contamination. The study by Garedew, et al. (2012), which states that traditional milking practices are likely to contribute to the contamination of the milk and proliferation of the microorganisms, is very much in support of the findings of the present study. *Escherichia coli* can easily be spread everywhere, including the milking parlor. Milking equipment may play a

significant role in the contamination of milk with this microorganism, mainly during milking, by means of direct contact between milk and the surfaces of contaminated equipment (Fagundes, et al., 2012). Majority of the farmers in the current study were using plastics utensils with small openings and traditional pots that are difficult to clean which can be easily contaminated with *Escherichia coli* , and these equipment can be important means of contamination to the milk through direct contact with milking equipment during milking or milk storage. Hence, in order to make the milk safe from being contaminated, it is necessary to always use clean hands and clean milking utensils during milking and during any other activities of milk production.

The present study permitted detection of the presence of *Escherichia coli* O157 in bulk milk samples collected from milk collection centers. It was found that the presence of *Escherichia coli* O157 is indicative of the fact that there is lack of proper hygienic milk handling procedures, poor source of water or lack of milking personnel hygiene. The observation made, during sample collection, while conducting the present study, clearly showed that the hygienic procedures were poor and the equipment used by the member farmers to deliver the milk were not properly cleaned; the possibility of this situation leading to the contamination of the milk by *Escherichia coli* O157 is very high. Several studies support this finding. According to Rahimi, et al., (2011) neglected sanitary measures adopted during manufacturing; handling and distribution of such fresh milk are the causative agents for the prevalence of *Escherichia coli* O157. Other findings by Giraffa, (2002) indicated that lack of proper animal and milk handling procedures; improper washing of milk equipment and ignorance of hygienic practices may be the possible reasons for the presence of *Escherichia coli* O157. Anand, et al., (2006), Gonzalo et al (2006) and Iyer, et al., (2010) also support the findings mentioned above. Transmission can also occur following direct contact with shedding animals (Chapman, 2000).

Another pathogen that frequently degrades the raw milk quality is the *Staphylococcus aureus* . It was observed that the *Staphylococcus aureus* was the other prevalent pathogenic bacteria detected in our study. The current finding is supported by Sim Kheng Yuen, et al., (2012) which revealed that high number of *Staphylococcus* count is found in a situation where there is no proper management and clean environment due to poor personal hygiene practices throughout the

milking process. Furthermore, the contaminated utensils especially the milking churns or any utensils that are in contact with raw milk might influence the microbial quality of raw milk along the milk chain. Milking personnel are the most probable sources of primary contamination of milk by *Staphylococcus aureus*. Poor personal hygiene and failure to maintain adequate sanitation and hygienic practices which are noticed during sample collection in the milk collection centers and indeed poor personal hygiene of the personnel working in any milk production process can play a damaging role through transferring *Staphylococcus aureus* to raw milk. Improper milking practices among the dairy farmers are also additional source of contamination of milk by *Staphylococcus aureus*. According to Daka, (2012) poor hygiene practices and improper milk collection hygiene contributed to the high proportion of *Staphylococcus aureus* in the milk. Mohamed and Fatima (2011) also indicated total *Staphylococcus aureus* count was high when cows were milked without applying hygienic practices and the counts decreased when hygienic practices were applied.

Lack of proper water for washing milk equipment and animal and ignorance of hygienic practices may be the possible reasons for high contamination of *Staphylococcus aureus* according to Giraffa, (2002). In the present study river was mentioned as source of water for some households, to which the contamination of milk with *Staphylococcus aureus* was attributed. Anand, et al., (2006), Gonzalo, et al., (2006) and Iyer, et al., (2010) also agreed on the fact that water used from river to wash the containers that were used for milking and to clean the udder may have contributed to the high level of *Staphylococcus aureus* contamination. Srinu, et al., (2012) further confirmed that possible contamination of *Staphylococcus aureus* could be due to water used in unclean farm conditions, unhygienic practices followed in the farm during milking and storage environment in the farm. In addition, the result of high contamination of *Staphylococcus aureus* at milk collection centers might be attributed to cross contamination of milk while bulking and poor handling across the dairy value chain (Desiss, et al., 2012)

Methicillin-resistant *Staphylococcus aureus* have been identified as a mastitis pathogen in dairy cows that can be isolated from bulk tank milk (Spohr, et al., 2011). In dairy cattle, the presence of MRSA could be due to the exposure of people working on dairy farms to the pathogen and the risk of the cattle getting

colonized or infected is high (Juhász-Kaszanyitzky, et al., 2007; Spohr, et al., 2011). The Second aspect is that the contamination of raw milk with MRSA can act as a route of transmission of MRSA to people consuming raw milk or as initial contamination in the production chain of raw milk products (Oliver, et al., 2009). Consumption of raw milk is common among farmers in the current study and in Ethiopia in general. The third aspect is poor animal health. Moreover, a study by Jones, et al., (2002) showed that most reported instances of foodborne MRSA have occurred through contamination by infected food handlers rather than the food itself.

In our study from all the *Staphylococcus aureus* identified, there were no positive MRSA; all were found to be sensitive to the test. This result in the current study indicates that Methicillin is not commonly used by the farmers to fight mastitis in dairy production in the study area. This is consistent with the study made by Daka, et al., (2012), which stated that Methicillin is not used on dairy cattle farms in the South Ethiopia. However, the negative MRSA result found in the present study doesn't mean there is no risk. Thus considering the effect of MRSA on the safety of milk, further research and multiple tests for MRSA should be done.

Recently the connection between MRSA, dairy cattle and people has been brought to attention; it indicates that the origin of bovine MRSA isolates is human. According to Lee, (2003), the rate of methicillin resistance among human *Staphylococcus aureus* isolates in Korea was higher and the incidence of MRSA in animals was low. The low incidence of MRSA in animals suggested that the animal isolates might have originally come from humans. A human origin was also suggested by the results of the study by Haenni, et al., (2010) and Turkyilmaz, et al., (2010). Considering these, further researches should be done on the connection between MRSA, dairy cattle and people; the study should not be limited to only milk contamination but also test should be done on those people who have contact with animals.

Brucellosis is considered a serious cause of productive losses in cattle. In addition, it is a zoonosis widely diffused all over the world. Generally, the susceptibility of cattle to Brucella abortus infection is influenced by age, sex, management and reproductive status of the individual animal (Radostits, et al., 2000). In our observation from the study, Brucella was the second identified as

an infectious disease. In the current study farmers indicated they prefer to own large number of cows to increase milk production. Sexually mature females are more susceptible to Brucella abortus infection than bulls. This susceptibility increases during pregnancy, and the animals get more susceptible with the advance of pregnancy. This might be one of the reasons why Brucella was found high in pregnant cows. In similar findings, it appears that female animals are generally more susceptible Brucella infection than the males (Keppie, et al., 1965) and (Yohannes, et al., 2012). Brucellosis is essentially a disease of sexually mature animals and susceptibility increases with sexual maturity and pregnancy (Radostits, et al., 2007) and Abraham Abebe, et al., (2008). Owning aged cattle can attribute to occurrence of Brucella. Age of animal has also been shown to be a risk factor for Brucella Uganda (Faye, et al., 2005) also in Ireland (Abernethy, et al., 2006) and (Mohammed, 2009).

In the present study area, purchasing favored animals is commonly done with the farmers; this could be one of the causes that increase Brucella contamination. Artificial insemination and multiple herds keeping i.e. sheep, goats and cattle also facilitate transmission of the disease Brucella. According to Yohannes, et al., (2012) and Holt, et al., (2011) contacts between cattle and sheep and goats were found to be the most primary risk factor for testing serologically positive for Brucella. Purchasing animals and artificial insemination have both been identified as risk factors for brucellosis (Stringer, et al., 2008). Poor herd management, feeding and lack of vaccination are also mentioned as factors to remission of Brucella. Aggregation and interaction of different animals at villages, grazing fields and water points, also facilitate transmission of the Brucella. The dynamics and frequent migration of herds might increase the chance of coming into contact with other potentially infected herds (Muma, et al., 2007). Lack of vaccination, mixed farming and use of surface water for cattle and goats have been noted as some factors that influence the presence of the disease in the area (Robert, 2012)

Use of river water, lack of hygiene during both milking time and milk collection provides an ideal environmental condition for the growth of *Listeria*. The organism can grow in soil, muddy and dusty conditions; it can also grow in water, dams, and in poorly managed feeds (ADASC, 1999) and (Tasci, et al., 2010). This gives the right picture and real explanation for the incidence of

Listeria observed in the current study where, for some of the respondents, source of water is river, poor milking is practiced, ways of transportation are traditional and lack of hygiene during milk collection prevails. As stated by (Bell and Kyriakides, 2002) product contamination by *Listeria* spp. occurs from either the environment or direct product contact to utensils and milking equipment and also from poor personnel handling practices. According to Faber and Peterkin, (2000) raw bulk-tank milk contaminated with *Listeria* is most likely done through environment polluted with feces/manure. The organism can be transmitted to cows via feeds such as improperly fermented silage and other feedstuffs, causing infection in the animal. Ragimbeau and Malle, (2001) also indicated that primary sources of *Listeria monocytogenes* in milk and dairy products feed are impure bedding, vegetation, soil, animal faeces as well as contaminated water, unclean udders, unclean human hands and handling equipment. An investigation of farm management practices found that no access to pasture, feeding poor quality silage and storing silage in a bunker significantly increased the risk of faecal shedding of *Listeria* in cattle (Nightingale et al 2005). According to literature data by (Fenlon, (1986), *Listeria* was isolated in higher percentage in samples from poorly prepared quality silage Tasci, et al., (2010). In the studies of (Vilar, et al., 2007) also *Listeria* spp. was isolated in samples of milk collected from cows fed poor quality silage.

The incidence of *Listeria monocytogenes* in raw milk could also be partly due to lacking in cooling facilities and poor unrefrigerated type of milk transportation equipment. Most of the dairy milk producers in the current study are smallholders, who do not have refrigeration systems and they have to transport their milk to the nearby Milk Collection Centers using plastic milking equipment. Latorre, et al. (2011) presented evidence that a source of *Listeria monocytogenes* contamination was unclean milking equipment. The study by Conly and Johnston, (2008) confirmed that *Listeria monocytogenes* can survive for longer period at low temperatures and on process equipment, and that bacteria has the ability to survive on the equipment used in milk production. In studies by Pan et al. (2006) it is stated that strains of *Listeria monocytogenes* were isolated from equipment used in food industry, which survived on the equipment and showed high resistance to sanitation measures, with the ability to act as constant source of contamination. Similar finding from Vazquez-Villanueva, et al., (2010) indicated that predominant and persistent strains of *Listeria* might be more adapted to the specific ecological environment of the milking system. Other risk

factor associated with contamination of raw milk with *Listeria monocytogenes* includes geographic location and seasonal variation. Incidences of *Listeria,* as reported by Ryser and Marth, (1999) show that the highest rate of *Listeria monocytogenes* and *Listeria innocua* contamination typically occurs during spring intemperate climate. Fentahun and Fresebehat, (2012) also indicated *Listeria* is most prevalent during spring and winter seasons. On the other had Nightingale, et al (2004) mentioned contamination of *Listeria* in winter was common than the other seasons.

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CHAPTER 8

Conclusion and Recommendations

8. Conclusion and Recommendations

Conclusion

Milk, as a harbor for a variety of microorganisms, it can be a disturbing source of food-borne pathogens. The presence of food-borne pathogens in milk can be due to direct contact with contaminated sources in the dairy farm environment and due to excretion from the udder of an infected animal. Milk and other traditional dairy products are important part of the Ethiopian food. However, the sanitary measures taken at the smallholder level and at the milk collection centers are generally unsatisfactory and cause deterioration and contamination of the milk and milk products.

The present study deals with the bacteriological-hygienic quality, particularly with pathogenic microorganisms of raw bulk milk from milk collection centers. The dominant bacteria in the raw milk samples in the study area are the following: *Escherichia coli,* Brucella, *Staphylococcus aureus* among the isolated species. E coli O157 and *Listeria monocytogenes* are also identified. The presence of these pathogens in milk indicates that contamination comes from various sources such as wastes from animal and human as well as from unclean environment and utensils. The high numbers of the isolated microorganisms not only contaminate the milk but also multiply and grow in the milk. The occurrence of pathogens is likely to affect the keeping of quality and safety of raw milk as well as products derived from it. The presence of these pathogenic bacteria in raw milk is of public health concern since drinking raw milk is still considered good for health in rural population of the country.

The sources of contamination are manifold. Starting from the milking procedure, bacteria enter into the milk from the cows' udder or the milk handlers' hands. The sanitation procedure is low to clean the udder or wash the hands thoroughly before milking; the use of disinfectants is not practiced and, in most cases, the water used is not adequately clean. Another serious factor throughout the milk chain is the milking and transport/storage equipment. Plastic buckets (jerry cans) are the commonly used containers for milking, storing the milk and transporting it. The problem with these jerry cans is the difficulty to clean them properly as the opening is too small to enter with hands or cleaning tools. The delivery of milk at the milk collection centers is done by using cups; the cups are considered to be a good measuring tool by the farmers. The cups, which are used the whole

day, in most cases, become a likely source/cause for contamination, as they are not properly cleaned. These, all the more, will increase the bacterial contamination of the products throughout the day. In addition lack of knowledge of good hygiene and not practicing it contributes to the poor quality of the milk.

The quality of milk produced and channeled to the processing plant from the dairy cooperative in the study area was found to be substandard. This is caused by the poor health of dairy herd, unhygienic conditions of milking, milk storage processes and the act of transferring milk into different containers in a manner that lacks sanitation. All these and the use of unclean milk equipment and inadequate as well as unclean water were factors that made the milk quality substandard. In addition, the river water used for cleaning is not of the required standard in terms cleanliness and germ-free, thus it contributes to making the milk in the area poor in quality. Furthermore, transportation is one of the major problems in the dairy sector. Some farmers transport milk to the collection centers on foot, while others use pack animals or public transportation. In such transportation, especially when they travel for long distances to reach the collection centers and the processing plants, the milk easily gets exposed to more contamination. Yet another serious problem for milk contamination is the fact that the milk is transported to the collection centers and the processing plants under high ambient temperature, using equipment which is not airtight, in a situation where there is no cold chain facility. All these negatively affect the milk and make its quality below standard.

Recommendations

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

- ✓ In order to improve milk hygienic quality and its shelf life, awareness should be created among milk producers and milk collectors as to the importance of adequate udder preparation, using hygienic milking technique, use of clean dairy equipment, washing of utensils and milk handler hands, and using clean water. Moreover dairy producers should be supported by services related to feed supply, veterinary, AI and other extension services for better milk production.

✓ In the present study, *Staphylococcus aureus*, *Escherichia coli* 0157 prevalence in milk originates from contamination due to unclean milking practices under hygienically poor situations. This shows the possible contamination of milk during the time of milking and during the process of transferring it from people to people or from place to place. Improving the hygienic conditions of the milking environment and utensils will reduce the prevalence of S. aureus or *Escherichia coli in* milk.

✓ The cows' teats and the milkers' hands should be washed, with clean water, carefully before milking starts. All containers used for storing and transporting milk should be cleaned each time milk has been emptied so as to prepare them to be used again. So, in order to ensure the cleanness of all milk containers, it is essential that training on milk hygiene to milk producers and milk collectors, should be provided on regular basis. The Training on keeping the environment of milking and passing over the milk to others clean is equally important. Training is very important to help people change something they are used to doing. Hence, as one of the best ways to change the outlook and the long time learned ways of doing things of both individuals at the cooperative centers and the farmers, training becomes imperative.

✓ The types of milk containers used also play a big role in impacting on the cleanliness and quality of the milk. It is recommended that using clean metal containers is much more hygienic than using plastic bags

✓ The absence of cold chains facility is also a factor that contributes to the low quality of raw milk. Provision of efficient milk cooling system at affordable prices at the collection centers and during transportation is very essential for significantly improving the quality of milk.

✓ Since there is a long interval between milking and delivery of the milk to the collection centers, providing cooling system, for the interval, should also be given due attention.

✓ In order to wash and keep the cows' teats and the milkers' hands as well as all containers used during milking and storing and transporting milk, for

effectively cleaning and sanitizing of milk equipment and udder preparations, clean and uncontaminated water should be available. In a situation where clean water may not be available, very well heat-treated water should be used for such purposes. With all these provided, all containers used for storing and transporting milk as well as for other uses in dairy process, should be cleaned each time milk has been emptied, so as to make them safe to be used again.

✓ The Government needs to assist and encourage establishment of laboratories to help determine quality of milk supplied by producers to the milk collection centers. Quality based differential payment should also be introduced based on compositional and microbial quality of milk. Such payment will create a competitive spirit among farmers and other milk producing agencies and try to outshine the market in producing better quality milk. This, in turn, will have positive effect in the production of quality milk among farmers leading to the improvement of the quality of locally manufactured milk and milk products.

✓ In addition the government should put regulations in place that deal with the sanitary operation in dairy industries to be consistently supervised by public health experts. The duties and responsibilities of the health experts is to remain vigilant to ensure that all measures are taken to prevent the multiplication of pathogenic microorganisms during the handling and processing of milk and milk products. This is considered to be very instrumental in preventing any contamination and pathogen-associated illness.

✓ Training courses and assistance need to be organized for milk producers and collectors and others working on dairy milk. The training should focus both on awareness raising and on practical and hands-on practice and exercise; this will help the farmers and other agencies producing milk to consciously act and produce milk of good and standard quality consistently.

✓ Further investigation is recommended for more clear and deep understanding of the contamination problems in the milk chain and to identify contaminants at species level by giving due attention to those pathogens of milk spoilage and that have human health hazard.

CHAPTER 9

Published Works

9. Published work

Fisseha, K. K. Jurgen, G. Mekonnen, H. Abebe, T. Hiwot, D. (2012): Dairy value chain analysis in Arsi zone, Ethiopia. 2nd Organic Animal Husbandry Conference: Tackling the Future Challenges of Organic Animal Husbandry. Hamburg, Germany, 12-14 September, 2012, Pp: 51-58.

Kebede, F.K. Hailemariam, M. Greiling, J. Abebe, T. Hiwot, D. (2013): Evaluation of Physicochemical Characteristics and Microbial Load of Raw Milk in Arsi Zone, Ethiopia. 16th International Congress on Animal Hygiene: Animal Hygiene, Health and Welfare as Corner Stones of Sustainable Animal Production. Nanjing, China, 5-9 May, 2013, Pp: 440-441.

CHAPTER 10

Annexs

10. Annex

Annex I Questionnaire format used

Part I. General information

1. Name of producer: ---

2. Name of the kebele: --

3. Name of the village: --

4. Name of the milk cooperative: --

5. Membership ID: ---

6. Farm owner's   sex: --------------------   age: ---------------------

7. Number of farm family members: total _____ adults _____ children____

Sex	Number under age group (years)				
	< 5	6 – 10	11 – 15	16 – 65	> 65
Male					
Female					

8. Religion
   a. Orthodox   b. Catholic   c. Protestant d. Muslim   e. other

9. Experience in dairy farming (in years): _____

10. Is agriculture your fulltime activity?   a. yes   b. no

11. Do you have someone to follow up the farm when you retire?
   a. yes b. no

12. Indicate the reasons why you produce milk?
   a. For sale and household consumption
   b. For household consumption only
   c. For sale only

Part II. Dairy farm management

13. Dairy herd structure

Category	Local	Crossbred	Total
Bull calf (<6 m)			
Heifer calf (<6 m)			

Heifers (6 to 18 m)			
In-calf heifer (>18 m)			
Milking cows			
Dry cows			
Bulls			
Oxen			
Total			

14. What kind of feeding system do you use? a. Grazing   b.   In   door feeding

15. Are you willing /able to invest in more animal feed in order to increase the quality and quantity of your milk? Yes/ no, why? _____

16. Which of the following feed types do you use to feed your dairy animals?

a. Stover   b. Mineral block   c. Bran   d. Oil-seed-cakes   e. Hay   f. Local brewer's yeast   g.   crop   residue
        e. other

17. What is your water source?
    a. Well
    b. River
    c. Tap water

18. How   often   do   you   give   water   to   your   dairy   animals? _____

19. What   is   the   age   at   first   calving   at   your   farm (months)?_____

20. What   is   the   calving   interval   at   your   farm   (months)? _____

21. What   is   the   annual   mortality   rate   at   your   farm? _____

22. Which animal diseases do you often encounter?
    a. Black leg
    b. Anthrax
    c. Parasites
    d. Rabies

   e. TB

   f. Brucellosis

   g. Mastitis

   h. Other

23. Do you have access to veterinary service? a. Yes b. no

24. If yes, who provides the service? a. Cooperative b. government
  c. private

25. Do you have to pay for the veterinary service? a. Yes b. no

26. Where do you buy vet drugs? a. Cooperative b. government c.
  private

27. Do you have access to AI service? a. Yes b. no

28. If yes, who provides the service? a. Cooperative b. government
  c. private

29. Do you want to replace your local cows with crossbreds? a. Yes
  b. no

30. Why?

31. If yes, how do you intend to do this?
  _____

Part III. Milk Production

32. How often do you milk your local cows? a. Once b. twice

33. How often do you milk your crossbred cows? a. Once b. twice

34. What is the average daily milk production of your local cow?

35. What is the average daily milk production of your crossbred cow?

36. Which family member is involved in the dairy farm work?

  ➢ Milking   male   female   children

  ➢ Feeding   male   female   children

  ➢ Processing male   female   children

  ➢ Marketing male   female   children

  ➢ Herding   male   female   children

  ➢ Other   male   female   children

37. Do you keep records of milk production? a. yes b. no

38. Can the family members mentioned above read and write?

  ➢ Male a. yes b. no

  ➢ Female a. yes b. no

  ➢ Children a. yes b. no

39. Do you process milk? a. Yes b. no

40. What kind of milk processing method do you use?
_____

Part IV. Milk quality issues

41. Did you receive training on clean milk production?   a. Yes   b. no

42. If yes, who provided the training?   a. Government   b. NGO   c. Cooperative

43. Have you implemented/practiced what you learned?   a. Yes   b. no

44. Do you wash your hands before milking?   a. Yes   b. no

45. Do you wash the udder of the cow before milking?   a. Yes   b. no

46. Do you use towel to clean the udder?   a. Yes   b. no

47. Where do you milk your cows?   a. Indoor   b. outdoor

48. What is your milking vessel made of?   a. Plastic b. metal c. traditional

49. How many times per day do you deliver milk to the MCC? _____

50. If you deliver milk once a day, how do you take the AM and PM milk?
a. Mixed b. separately c. PM only   d. AM only

51. Do you encounter milk rejection due to inferior quality milk?   a. Yes   b. no

52. If yes, how often in a month?
   ➢ During the dry season   _____
   ➢ During the wet season   _____
   ➢ During fasting season   _____
   ➢ During non-fasting season   _____

53. Did you receive training on dairy animal husbandry?   a. Yes   b. no

If yes, who provided the training?   a. Government   b. NGO   c. Cooperative

Part V. General questions

54. Mention three of your main problems in dairy production and marketing activities. (Why we limit to three, let us make it open (without limitations)?

55. What should be done to solve these problems?

Annex II Field and Laboratory Work in Picture

Culturing samples

Reading results

Data collection/surveying

Milk Sample collection from PDC

Annex IIIArsi dairy union member primary dairy cooperatives

Name of PDC	Distance form Asella Town (km)	Number of Members		
		Female	Male	Total
Assela Town*	0	30	47	77
Bekoji*	56	7	77	84
Bilalo*	12	5	65	70
Dosha	5	6	41	47
Goba Lencha*	37	3	52	55
Gora Fana	3	0	11	11
Lemu Ariya*	45	20	65	85
Lemu Michael	47	16	58	74
Meraro	66	3	26	29
Total		90	442	532

*Selected PDC

Annex IV Ada primary dairy cooperatives member stations

Name of Station	Number of Members		
	Female	Male	Total
Lemlem*	62	56	118
Kebele 02*	111	103	214
Kebele 03	77	71	148
Kebele 08	30	33	63
Kebele 11*	58	80	138
Kebele 15	12	26	38
Around Vanjelica	25	19	44
Medihanealem	29	27	56
Kebele 01	9	10	19
Kebele 05	19	21	40
Denkaka*	3	20	23
Around ILCA	5	2	7
Babugaya*	13	1	14
Total	453	469	922

* Selected Station

CHAPTER 11

Acknowledgements

11. Acknowledgements