Microbiological Quality of Milk Produced in Urban and Peri-Urban Farms in Central Ethiopia and its Public Health Impact

THESIS

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Abstract

Ethiopia has a large potential for dairy development and much focus has been placed on market demand and productivity. As the industry grows, it is imperative to focus on food safety and quality standards that will increase productivity and trade capacity and improve public health. One active approach being taken is the implementation of a milk quality-based payment system. To help implement quality standards and determine the public health significance of milkborne pathogens, a pilot study was performed to establish baseline data on the microbiological quality of milk throughout central Ethiopia. Fresh bovine milk samples were taken from individual farmers (n=119) and combined bulk tanks (n=29) from collection centers in Selale, Asela, Akaki, and Debre Zeit to determine the prevalence of *Salmonella enterica* and *Staphylococcus aureus*. To further assess the public health impact, isolates of *S. aureus* were tested for antimicrobial resistance and the presence of enterotoxigenic genes. In addition, surveys were administered to participating farmers and collection center owners to gain an understanding of milking practices on urban and peri-urban farms in central Ethiopia and to determine possible risk factors of milk contamination. *S. aureus* was present in 26.1% of pooled samples and 51.7% of combined bulk tank samples. Of the strains isolated from pooled milk, 83.9% were resistant to penicillin, ampicillin, tetracycline, or a combination of the three with the highest proportion resistant to penicillin. Enterotoxigenic genes were found among 25.8% of the isolates with *sea* accounting for the highest proportion. The pulsed field gel electrophoresis (PFGE) analysis resulted in three different clusters with clonal relatedness between strains within geographical locations. We found a 0% prevalence of Salmonella in both pooled and bulk samples.
tank samples. There were very few differences in milking behaviors between regions. Three factors associated with recovery of *S. aureus* from milk are; administering a pre-test to new cows prior to entering the herd, high levels of production, and assigning a specific individual to only milk. It is clear that *S. aureus* is present in a significant proportion of milk produced on urban and peri-urban farms and poses a potential public health threat due to the presence of staphylococcal enterotoxin (SE) genes. Determining and implementing quality standards for a quality-based payment system is one step towards encouraging farmers to produce quality milk and in turn improve public health. It is also essential to ensure farmers have the knowledge and resources to meet the standards set forth.
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Introduction

Ethiopia has a large cattle population with more than 50 million head, the largest in Africa, and holds great potential for dairy development\(^1\). In the capital city Addis Ababa alone, there are more than 5,200 dairy producers with more than 58,000 head of cattle\(^2\). While the industry is growing at a rapid rate, no milk quality standards currently exist. Therefore, it is important to establish milk quality standards that focus on food safety measures in order to improve public health. Baseline information on foodborne pathogens is important in setting legislative standards for milk quality and in potentially implementing a quality based payment system that is currently under consideration by the Ethiopian government. The following are the goals and objectives of the project:

1. To obtain baseline information on microbiological aspects of milk quality by determining the prevalence of *Salmonella enterica* and *Staphylococcus aureus* in fresh bovine milk samples collected from pooled on-farm samples and bulk tank milk.
2. To determine the public health risk presented by milk produced in central Ethiopia through measuring antimicrobial resistance (particularly MDR-SE and MR-SA) and carriage of antimicrobial resistance genes against commonly used antimicrobial classes.
3. To determine if *S. aureus* isolates found in milk throughout central Ethiopia carry staphylococcal enterotoxin genes, particularly sea, seb, sec-1, sed, and see and the gene for toxic shock syndrome, tst.
4. To support biosecurity risk management in the milk value chain and determine potential transmission patterns of pathogens by assessing the clonal relatedness of pathogenic
strains. Genotypic relatedness of bacteria isolated from various geographic location and time periods will be assessed

5. To support policy standards for a quality-based payment system in the milk value chain and assess the potential public health impacts by providing comprehensive qualitative data on pathogen prevalence, phenotypic and genotypic attributes of the pathogens.
Literature Review

2.1 Introduction

The dairy industry is an important part of agriculture throughout the world and contains many unique attributes that play key roles in the production, transportation, and marketing of dairy products. The industry is continually changing and contains great potential for growth among developing countries due to an increase in demand for milk and dairy products. While this growth can improve the livelihoods of farmers through increased income and sustainability, it is essential to keep in mind the importance of producing a quality product that is safe for the consumer. Milk is highly nutritious and an important part of diets across the globe but is also a perfect medium for the growth of several pathogens of public health significance.

Ethiopia, with the largest livestock population in Africa, has experienced rapid growth in the dairy industry in recent years. As the industry continues to grow, focus needs to be placed on food safety measures to ensure a safe and high-quality product for the consumer. *Salmonella enterica* and *Staphylococcus aureus* are two examples of pathogens found in milk that account for a large portion of foodborne illnesses around the world.

2.2 Overview of the Dairy Industry

The dairy industry has several characteristics that set it apart from other areas of agriculture and contribute to how milk is produced, processed, transported, marketed and regulated. The Food and Agriculture Organization (FAO) places emphasis on four attributes that have helped shape the industry into what it is today. First, milk is a perishable, raw material that is produced every day and is bulky in regards to transportation. Because of the perishable nature, milk must often be processed into a more stable form, or transported quickly to a place.
where it can be refrigerated to slow and prevent bacterial growth. In addition, milk and dairy products have strict and comprehensive quality regulations in developed countries, and there has been a push for the same standards in developing countries.

The expenses associated with transportation and quality control lead to the second important attribute: the strong role of co-operatives in milk processing. In developing countries especially, thousands of small-scale dairy farms contribute to the total amount of milk produced and processed. The role of dairy co-operatives is to create economies of scale and reduce the overall cost associated with marketing and transportation. This is done through the bundling of services for a large number of dairies. When small dairies are part of a co-operative it strengthens their bargaining power when dealing with processors and can result in higher prices received by the farmers for their milk. Co-operatives also play an important role in coordinating veterinary services and providing training on ways to improve milk quality and production.

The third attribute relates to the unstable socio-economic position of dairy farmers. Because the production of dairy often depends on factors farmers cannot control, such as weather, market fluctuations, demand, and feed shortages, dairy farmers are more protected through tariffs and other regulations than other areas of agriculture. Both developing and developed countries have implemented policies to protect small farmers that often distort the dairy market at the same time. Surplus production is encouraged in developed countries through producer subsidies and then placed on the world market through government paid export tariffs. Because developed countries often have lower operating costs than developing countries, they are able to sell dairy products at a lower price resulting in what some call unfair competition. Import tariffs are prevalent in both developed and developing countries in hopes of protecting the small-scale dairy farmer from the competition of the world market. While the
effects are difficult to quantify, the distortion has affected the livelihoods of poor small-scale farmers throughout the developing world\textsuperscript{3,8,11}.

Finally, milk and milk products are extremely valuable but equally as expensive to process and distribute. Milk is a highly nutritious product that is important in the health and livelihoods of families throughout the world and is often processed into a variety of products to add value\textsuperscript{3}. These products include standardized milk, pasteurized milk, cream, skim milk, butter milk, cheese, butter, evaporated or condensed milk, milk powder and casein. The processing of dairy products can create a source of income and employment, but before a strong dairy industry can develop, adequate infrastructure needs to be in place. Transportation and low-cost refrigeration should be available as well as the technology to package and distribute the products efficiently while maintaining high-quality products that are safe for consumers. While there is potential for the dairy industry to grow and improve economic development in developing countries with ample milk supply, special measures need to be taken to ensure the industry is successful and profitable\textsuperscript{3,11}.

To accommodate these unique attributes and to account for increases in technology and globalization, the dairy industry has gone through many changes and seen a great amount of growth over the past several decades\textsuperscript{3}. Worldwide, total milk production has increased by 32 percent over the last 24 years. While this is a significant increase, population growth exceeds the growth in production which is demonstrated by a decrease in per capita world milk production. Most of the population growth has been in developing countries, where the production of milk and dairy products has been unable to keep up\textsuperscript{8}.

2.3 Developed Countries

In most developed countries the dairy industry is moving away from many small-scale dairies towards a small number of large dairy cow operations. In the United States for example,
the number of dairy cow operations decreased by 33% from 2001 to 2009. The trend has been an increasing number of large scale farms consisting of 1,000-3,000 dairy cows that operate at a lower cost than small-scale dairy farms. While the actual number of dairy operations is decreasing, milk production and number of dairy cows has been increasing. The majority of the increase in milk production is due to the improvement in productivity of dairy cows as opposed to the growing numbers of cattle. Since the 1970s, milk production in the U.S. has increased by almost half but the number of milking cows has decreased from nearly 12 million to 9 million. From 2008 to 2009, the U.S. reached a record high in productivity of an average dairy cow for the 7th year in a row. The increase in milk produced per dairy cow can be attributed to culling strategies, breeding for improved genetics, improved veterinary care services, and improved feed input.

Dairy breeds in developed countries have been genetically selected to produce high milk yield. In the U.S., 90% of the dairy herd is made up of Holsteins that produce large volumes of milk high in butterfat and protein. While these breeds are extremely productive, the associated costs with feeding the cattle are high for the farmer. Jersey cows make up 7% of the U.S. dairy herd and are becoming more popular due to their efficiency of milk production. They are much smaller than the Holstein and require fewer inputs to sustain growth and milk production.

Throughout the United States and many European countries, most milk is produced by cows raised in intensive production systems. Intensive systems include tie stall barns, free stall barns, and open lots. The feed source is typically high in concentrates and comes from pastures, rangelands, annual forages, and purchased feeds. Intensive production systems in general are characterized by a consistent and relatively high return per unit of input.

Pasture based systems are still used as well in developed countries. In New Zealand, for example, it is the primary means of raising dairy cattle. The focus in these systems is on the
productivity of the rangeland or pasture since grazing is the primary source of feed\textsuperscript{15,16}. Grazing is maximized when the climate permits for optimal growing conditions. Conserved feed is used for the remainder of the year\textsuperscript{15}. New Zealand has been a model for success in the dairy industry in using an almost exclusively pasture based system. A temperate climate with little variation throughout the year and predictable rainfall enables them to operate dairy farms with a much lower cost than the intensive based production system used in the U.S. and other developed countries\textsuperscript{18}.

To have a successful dairy manufacturing sector, it is essential to have an accessible and comprehensive transportations system, low-cost refrigeration technology, and the technology for processing and packing products in a hygienic manner\textsuperscript{11}. The strong infrastructure and technology present in developed countries allows for the hygienic and quality production of milk and dairy products that are safe for the consumer. In the United States, most dairy farmers adhere to milking procedures that promote hygiene. At a minimum they include cleaning the teats thoroughly and drying before milking. Once the cow is milked, the teats are disinfected with an approved teat dip to prevent the transmission of mastitis to the entire herd. The milk must then be cooled to 45°F within two hours of milking completion to prevent a significant rise in bacterial counts\textsuperscript{19,20}. Milk is often cooled via a plate cooler and then stored in a bulk tank where periodic temperature readings are taken either manually or automatically to ensure the proper temperature is maintained. Milk is transported between the farm, processing plants, and retailers by refrigerated trucks or insulated tankers. Samples from each bulk tank are taken and tested to ensure the milk is safe and of high-quality prior to processing\textsuperscript{19,21}. In order for farmers to receive a premium for high-quality milk, the milk must be free of antibiotic residues, have a somatic cell count (SCC) not exceeding 750,000 per ml, and a bacterial count less than 100,000
per ml prior to comingling with other producer milk and less than 300,000 per ml once comingled⁶.

In developed countries, only 5-10% of milk is actually consumed in its raw form. The majority of milk is processed into products with added value such as pasteurized milk, cream, cheese, butter, yogurt, ice cream, powdered milk, and other dairy products¹¹. The primary purpose of processing milk is to lengthen the shelf life and to significantly reduce the risk of transmitting diseases. Processing milk not only creates a safe product but also increases income and employment opportunities²⁰.

In addition to the ability to process enough milk to fulfill demand while maintaining a high-quality product, a strong marketing system must be in place to provide the proper dairy products in the correct amount and in the right place. As previously mentioned, dairy co-operatives can play an essential role in the marketing of milk. In the United States, 98% of farms are family owned and operated and the large majority of these farms operate through dairy co-operatives²². There are nearly 150 dairy co-operatives that account for over 80% of the milk marketed. While the number of co-operatives has been steadily decreasing over the years, the co-operatives currently active are handling larger volumes²³. Dairy co-operatives in the U.S. employ tens of thousands of people and consistently make a profit. In 2007, there was an overall 12.2% return on equity²².

The success of the dairy industry in the United States is in large part due to several different organizations working together to implement quality standards throughout the entire milk value chain. This ensures that milk and milk products are safe for the consumer³. Due to the importance of milk in the diets and nutrition of the population, especially young children and the elderly, the United States Public Health Service (USPHS) has taken special interest in milk sanitation since the early 1900s. In addition, milk can serve as a source of disease transmission
and has been the cause of many foodborne outbreaks in the past. Because of the perishable nature of milk and its ability to transmit zoonotic diseases, it is imperative to maintain quality management from the producer to the consumer.

In 1924, the USPHS developed the *Standard Milk Ordinance* for voluntary adoption by state and local milk control agencies to help plan, implement, and maintain effective programs to prevent the occurrence of milkborne diseases. This regulation is now titled Grade “A” Pasteurized Milk Ordinance (Grade “A” PMO) and has resulted from the continuous input from the Health and Agricultural Departments: all levels of the dairy industry to include producers, milk plant operators and equipment manufacturers: and educational and research institutes. The Grade “A” PMO is generally accepted as the national standard for milk sanitation and suggests regulations and standards on every aspect throughout the dairy chain, including production, transportation, processing, handling, sampling, examination, labeling, and sale of Grade A milk and milk products. It also gives explicit instructions on how to inspect dairy farms, milk plants, and other facilities that play a role in the overall milk production process. While it is not federally mandated to follow the guidance provided in the Grade “A” PMO, all 50 states, the District of Columbia, and U.S. trust territories have adopted the ordinance.

The Grade “A” PMO also incorporates the use of Hazard Analysis Critical Control Points (HACCP) as a means to maintain quality standards for milk and milk products. HACCP is a management system in which food safety is addressed through the scientific analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product. The concept behind HACCP is to provide a proactive approach where food safety hazards can be identified and fixed prior to any negative consequences. HACCP is a voluntary program, but is
internationally accepted as an effective way to control food safety hazards and is used in many dairy plants throughout the U.S\textsuperscript{6}.

2.4 Developing Countries

The picture of the dairy industry in developing countries is much different than what is seen in the developed world. Overall milk production has increased but only a small percentage is due to increased productivity of dairy cattle. Most of the increase is due to a rise in the number of milk producing livestock\textsuperscript{8}. Regardless, developing countries have been increasing their share of total worldwide milk production. From 1990 to 2000, China, Korea, select Southeast Asian countries, the Middle East and North Africa, and Central and South America doubled their combined share from 10\% to almost 20\% of the world’s milk supply. The increase was much less significant in South Asia and Sub-Saharan Africa (SSA) whose production grew from just under 20\% to slightly over 20\%. Sub-Saharan Africa’s share of worldwide milk production has remained at approximately 3\% from 1990 to 2000, showing that the dairy industry is not expanding there as much as in other developing countries\textsuperscript{11}. Additionally, studies comparing SSA to other developing countries found that SSA had the lowest milk production per cow and the smallest increase in the proportion of cows milked from 1985 to 1998\textsuperscript{24}.

Despite the slow growth seen in developing countries, the International Livestock Research Institute (ILRI), along with other agencies, projected that the demand for dairy and dairy products will more than double in developing countries by the year 2025 with an estimated annual growth in consumption of 3.3\% per year\textsuperscript{25,26}. This period of growth is referred to as the Livestock Revolution and is based on the projected increase in demand through population growth, urbanization and increased income generation. With this growth there will be changes in eating habits. As incomes rise, more animal products will be consumed to increase nutrition. Urban populations are also more likely to include milk and meat products in their diets based on
their preference for increased variety and convenience. The rate of urbanization of developed countries collectively averaged 3.1% per year between 1970 and 1995 with similar trends predicted into the future.26

In order to keep up with this increase in demand, there has been a push to focus on a more productive and market oriented dairy system throughout developing countries. A variety of international organizations such as Land O’Lakes, Send a Cow, and Heifer Project International have been working to promote milk production in SSA with the objectives of improving nutrition through increased milk consumption and increasing income generation for smallholder dairy farmers. There are many constraints and challenges involved with promoting dairy development in developing countries. The main areas of focus have been on genetic improvement, promotion of marketing and consumption of milk and dairy products, veterinary extension services, credit and farm inputs, and import policies. Very little focus has been placed the quality of the product being produced.

2.5 Dairy Industry in Eastern and Southern Africa

Milk production systems in Africa consist of pastoralist herds, herds kept by agro-pastoralists, and crop livestock farmers. Traditional systems characterized by subsistence farming where only enough milk is produced to support the producer and neighbors is predominate throughout the continent. This is also referred to as the informal market and remains the most important outlet for milk in Africa.27 More than 70% of milk and milk products are consumed on the farm or sold through the informal market. This system is unregulated and often provides low quality milk that poses substantial health risks to the consumers. A market-oriented system would enable quality standards to be implemented more easily and would improve farm income, create employment, and contribute to food security in rural areas.20 However, the success and benefits of marketed milk is dependent on the surplus produced over
what is consumed by the producer and neighbors and the accessibility of urban markets\textsuperscript{28}.

Transporting perishable dairy products to urban consumers is very expensive and most countries throughout Africa lack a strong infrastructure and refrigeration capabilities, making it difficult for small-scale producers in rural areas to participate in the dairy market\textsuperscript{11,20}.

Cattle are the source for more than two thirds of the milk in Africa and also produce most of the marketed milk throughout Eastern and Southern Africa. The majority of marketed milk is produced through integrated crop-dairy systems concentrated near urban consumption centers\textsuperscript{27,28}. The potential for pastoralist and agro-pastoralist systems to compete in a market depends largely on their distance from urban consumption centers, which determine the cost of collection and transportation\textsuperscript{27}.

Between 1990 and 2004, the demand for dairy products in Africa increased on average 4.0% per year while the growth in production was only 3.1%. Based on the demand, there is a need to improve and increase production throughout the dairy industry. The total bovine milk production in Africa in 2004 was 22,244,474 tons coming from a total of 46 million dairy cows. This gives dairy cows in Africa a productivity rate of 461Kg per cow per year which is only one fifth of the world’s average\textsuperscript{5}. Tickborne diseases and trypanosomiasis often restrict the upkeep and production of cattle in the humid regions of Africa. Therefore, dairy systems are more prevalent and productive in the highlands, the wetter semi-arid and drier subhumid areas, and closer to or within urban centers\textsuperscript{27}. While the general growth of milk production in Africa has been slow, some countries have seen more rapid growth in the volume of bovine milk produced\textsuperscript{5}. Eastern Africa accounts for most of the bovine milk produced within Africa. Kenya, Sudan, Ethiopia, Somalia, and Tanzania are the top five producers and account for two thirds of the bovine milk on the continent\textsuperscript{24}. Kenya has seen much success in its milking industry and has been a model to surrounding countries, especially Ethiopia.
2.6 Dairy Industry in Kenya

While many countries in SSA had a decrease in per capita milk production, Kenya’s production increased from 1985-1995. At 85kg (82.6 liters if use specific gravity of 1.029) per capita, the availability was four times greater than in Tanzania and Ethiopia. The importance of milk in the diets of most Kenyans combined with the growing population, urbanization, and purchasing power has contributed greatly to the increased demand for milk and dairy products throughout the country. In 2006 milk consumption per capita increased to 145 liters, which is 5 times greater than other East African countries. Kenya is also one of the few African countries that produces enough milk to support the rapid population growth of the country.

Over the past several years, Kenya has adopted many policies to support the small-scale specialized dairy production system. Prior to 1992, the dairy industry in Kenya was controlled by the government and consisted mainly of large scale farmers and one processing plant, Kenya Co-operative Creameries (KCC), who enjoyed a monopoly in the marketing and processing of milk and dairy products. During this time, an infrastructure was put in place for dairy farming that supported large scale settler farmers but neglected the smallholder dairy farms. The role of the smallholder farmer began to be addressed in the 1960s by the Kibaki Commission on Dairy Development. KCC was mandated to provide increased access to its processing plants and ensure a market for all raw milk. The expansion of KCC led to the introduction of more chilling stations and processing and packaging plants throughout the nation. At the same time, the government was investing in the widespread use of improved dairy breeds, animal health services and training programs. Land from large farms previously owned by white settlers was redistributed and by the mid 1970s, smallholder dairy farmers produced the majority of the milk in Kenya. Eventually, KCC began to struggle and there was a push to end the monopoly.
After 1992, the government liberalized the dairy industry. Private investments in milk marketing and processing were encouraged while producer and consumer prices were deregulated. The new approach stemmed competition. In 2004, there were 34 processors in operation that collectively processed 1,000,000 liters of milk per day and had the capacity to process over 2.5 million liters per day\textsuperscript{29}. By 2005, 14% of the total milk supply was processed through one of the 40 registered processors\textsuperscript{29,31}. The milk is processed into pasteurized fresh milk, yogurt, mala (sour milk), ice cream, cheese, Ultra High Temperature (UHT) milk, powder milk, butter, ghee, and condensed and evaporated milk\textsuperscript{29}.

An overwhelming portion of the dairy industry is dominated by smallholder producers, and the informal market provides 86% of marketed milk to consumers\textsuperscript{29,32}. While the new policies encouraged privatization of the processing and marketing of milk, the importance of small-scale milk vendors and the informal market could not be ignored\textsuperscript{33}. By the end of 2000, there were 1500 licensed informal milk traders that consisted of small-scale producers, milk bar operators, milk transporter traders and mobile milk traders\textsuperscript{29,33}. In 2004 the Smallholder Dairy Project (SDP) spearheaded policy changes that promoted small-scale milk vendors and streamlined the process for acquiring licenses. Through extensive research, the SDP was able to show the importance of small-scale milk vendors and the informal market. Previously, the process for obtaining the proper license to market milk was difficult, and many small-scale milk vendors operated illegally. Kenya Dairy Board officials implemented training and mandated requirements for certification and licensing\textsuperscript{33}.

The SDP also conducted research on quality and safety issues throughout the informal market. At the same time procedures for licensure were made more feasible, the Kenya Dairy Board and public health officials were required to ensure that licensed milk vendors meet quality standards for hygiene. Field workers are now required to perform spot checks, field
visits, and training to ensure milk vendors adhere to proper testing, sanitation, and health requirements. Field workers also provide advice on proper food safety measures and have the authority to issue milk movement permits.

While the formal market is profitable and growing, it is difficult for processors to compete with the informal market due to the higher prices this outlet can provide to small-scale farmers. Consumers can purchase milk and dairy products at lower costs through the informal market, and many Kenyans prefer the taste. Recently, however, the processing sector has changed its marketing strategy to focus on the safety and value of pasteurized and packaged products and to embrace small-scale milk vendors. As the population continues to grow and become more urbanized with consumers gaining a greater purchasing power, the demand for high-quality and safe dairy products is expected to increase.

2.7 Dairy Industry in Ethiopia

Ethiopia is similar to Kenya in many regards and holds potential for the same success throughout the dairy industry. The country maintains the largest cattle population in Africa at 50.8 million. Of these, 7.4 million are dairy cows who’s primary purpose is for milk production and 10.7 million are milking cows - any cows that are used for milk regardless of their primary purpose. Milk and dairy products play a significant role in the nutrition and health of Ethiopians. Animal products account for 8% of the total food consumption in Ethiopia and approximately 50% of this is accounted for by milk and dairy products. The dairy industry in Ethiopia has grown significantly in the past few decades and possesses the potential to expand at a greater rate in the future.

Between 1999 and 2009, milk production increased by 500,000 tons - a 42% increase. From 1966 to 2000 the increase in production was, on average, 1.6% per year. Even though there has been a large and consistent increase in production, the dairy industry has generally
not been able to keep up with the rapidly expanding population with a 3.0% per year growth rate until recently\textsuperscript{37}. The per capita consumption has declined significantly from 28 liters per capita per year in the 1980s to 16 liters per capita per year in 2000\textsuperscript{27}. The world average per capita milk consumption is 100 liters while in Africa the average is 27 liters. Ethiopia’s consumption rate is well below both of these\textsuperscript{38}. Even so, the political changes in the 1990s associated with the Ethiopian People’s Revolutionary Front have stimulated a higher growth in the dairy industry than has previously been seen. Taking the average of just the decade following 1993, milk production increased at a rate of 3.0% per year. Based on the country’s livestock potential, favorable climate, continual increase in population and urbanization, and market-friendly policy reforms supporting dairy development, the dairy industry in Ethiopia is expected to continue to grow over the next decade\textsuperscript{39}.

In order for the dairy industry in Ethiopia to continue to grow, focus needs to be placed on both demand and supply inputs. The demand for milk products in Ethiopia is expected to increase considerably, with a large portion coming from urban consumers. The urban population accounts for 17% of the total population and is predicted to maintain a 3.8% rate of urbanization per year between 2010 and 2015\textsuperscript{37,40}. Additionally, while Ethiopia has one of the lowest per capita GDP in the world, ranked 214\textsuperscript{th} in the CIA World Fact Book, the per capita GDP has grown over the past few years from 900 USD to 1000 USD\textsuperscript{37}. Population growth, urbanization, and increase in purchasing power are not the only factors that need to be considered when dealing with demand. Ethiopia has a unique attribute in that 43.5% of the population is Orthodox Christian who abstain from all animal products between 180 and 250 days per year\textsuperscript{41,37,42}. A study done among consumers in Addis Ababa showed that dairy consumption throughout the city decreased by 60% on fasting days. This is one reason suggested to explain the low per capita dairy consumption in Ethiopia\textsuperscript{42}.
It is clear that the demand for dairy products will increase, but production needs to increase at the same rate for the dairy industry to see further development. As far as supply, focus is placed on improved dairy breeds, veterinary services, food and forage inputs, access to markets, and quality management. While Ethiopia maintains a large population of dairy cows, only 13% are milked. Kenya has a similar number of dairy cows, but 33% of them are milked. The overall productivity of dairy cattle increased from 1985 to 1995 by 17% but is still much lower than other countries in the region. The low average yield is in large part due to low numbers of exotic dairy cattle and crosses. Improved cross breeds account for only 1.8% of the total milking cow population and 47% of these are in Addis Ababa. The local zebu cattle that make up the large majority of the cattle population produce 100 to 200 liters of milk per cow annually, which is only a fraction of the milk produced by cross bred cattle - 1400 to 1700 liters per cow per year.

While many European breeds have been imported - the majority being Fresian and Jersey - it is important to conserve the genetic traits of indigenous cattle that provide disease resistance, heat tolerance, and the ability to use poor quality feed. In 1981, the Ethiopian government established the National Artificial Insemination Center (NAIC) along with several other centers that specialize in improving specific breeds. The NAIC keeps 40 bulls for semen production to help with artificial insemination (AI) and inseminates close to 40,000 cattle per year, mostly in Addis Ababa. The centers for breed improvement focus on introducing traits that will optimize production while conserving traits that enable dairy cattle to thrive in harsh environments. The AI services and breed improvement programs are under government control and suffer from limited focus on genetic improvement, lack of selection criteria for bulls, lack of pedigree information, and low efficiency and effectiveness of AI technicians.
Veterinary services are also controlled mostly by the government. Poor health of dairy cattle can directly affect productivity through death, weight loss and poor fertility\textsuperscript{1}. Poor livestock health can also lower the quality of the milk produced and introduce pathogens of public health significance into the milk. The government run programs focus on disease surveillance, eradication campaigns, vaccine production, drug and vaccine quality control, quarantine, and food hygiene inspection measures\textsuperscript{10}. According to the Central Statistical Agency of Ethiopia(CSA), 32.6\% of cattle receive one or more vaccines against various diseases, including anthrax, black leg, pleura pneumonia, and hemorrhagic septicemia. Additionally, of the 8.96 million cattle that CSA projected to be diseased, only 57\% were treated (which, however, is significantly greater than the 22\% treated when talking about diseased livestock of all types - 46.2 million animals in total)\textsuperscript{1}. As shown by the low percentages of livestock reached, there are several obstacles in distributing sufficient veterinary care. These include low manpower, lack of adequate transportation, availability of drugs and other supplies, poor information, limited communication and reporting systems, and financial concerns. This is also an area where the private sector is hesitant to invest due to lack of capital and high operating costs. Livestock keepers are unwilling to pay for treatment and can often find cheaper treatments from illegal markets. Non Governmental Organizations (NGO) also often provide subsidized medication that further discourages investment from the private sector. The control of zoonotic and foodborne diseases is very weak. In addition to the limited reporting systems, livestock owners are unlikely to report symptoms due to a high cost of treatment or the possibility of culling their livestock for certain zoonotic diseases. Some co-operatives take part in providing veterinary services at an affordable price\textsuperscript{10}. This is a promising strategy that could improve the health and productivity of livestock while helping to ensure quality products and improving public health.
In order for farmers to have an incentive to produce a surplus, there needs to be a profitable outlet for their product. Since the Dergue regime was overthrown in 1991, policies have been in effect that promote a market-oriented dairy industry and push to enable smallholder dairy farmers to take advantage of the market. While land is still owned by the government, tenants are given more security with the right to inhabit land indefinitely, pass land onto children, and to temporarily lease the land. Most importantly, policies allowing land re-distribution were abolished. Specialized projects such as the Smallholder Dairy Development Project (SDDP) began to provide small-scale dairy producers with incentive to produce more through increased market access. Through the SDDP, 30 co-operatives were developed around Addis Ababa whose purpose was to serve dairy farmers rather than govern as they did during the Dergue Regime. Many other specialized projects are being implemented throughout the country, tailoring their objectives to the different regional characteristics. In addition to the specialized projects, efforts are being made to improve veterinary and breeding services and the promotion of forage and feed.

2.7.1 Dairy Farming Systems in Ethiopia

Agriculture is an extremely important part of Ethiopia’s economy. Currently, the agricultural sector accounts for 45% of the total GDP and 85% of the total employment. The different farming systems in Ethiopia vary based on geographical locations and access to markets. They can loosely be categorized into four main systems, although there is variation within each of the systems. The systems are a small commercial system, pastoral and agro-pastoral systems, smallholder mixed farming systems, and peri-urban and urban systems.

The small commercial farming system consists of large private and state farms and accounts for less than 0.03% of the country's milk production. The pastoral/agro-pastoral system, are located in the lowlands and account for 22.4% of total milk production.
Smallholder mixed farming systems account for the majority of the milk produced (63.3%). These systems use mostly local breeds and are located in the highlands. The indigenous breeds of zebu used often require limited input but are low-producing. The cattle are traditionally managed, meaning they feed from native vegetation, aftermath grazing, and crop residues. Both the pastoral/agro-pastoral and mixed livestock producers are part of the rural dairy system. The rural dairy system is a non-market oriented system where most of the milk produced is consumed at home. The surplus is sold to neighbors or processed into traditional products to enter the informal market.

Urban and peri-urban systems are located close to urban centers, have limited available land and have access to co-operatives, collection centers, and the market. They use a combination of local and cross-bred cattle and provide 14.3% of the total milk production. The majority of the milk produced by these systems supplies Addis Ababa and regional towns. There are over 5,100 small- and large-scale urban dairy farmers in and around Addis Ababa. The urban milk system produces approximately 35 million liters of milk per year. Of this, 73% is sold, 10% is kept for home consumption, 9.4% is fed to calves, and the remaining is processed into butter or ayib (cheese). While the majority of milk is sold, 71% is done so through the informal market.

2.7.2 Marketing and Processing of Dairy Products

With regards to the marketing situation, 97% of milk produced is consumed through an informal market where milk is either consumed in the home or sold directly to the consumer as raw milk or traditionally processed products. Milk sold through the informal market is not pasteurized, can easily be adulterated, and poses health risks to consumers. This system is difficult to regulate, and no current standards for milk quality exist. The informal system continues to dominate the dairy industry in Ethiopia because many factors make it difficult for farmers to participate in a market-oriented system. The lack of infrastructure creates high
transaction costs associated with transportation of milk and dairy products to processing plants and urban centers. The informal market does not incur these additional costs resulting in higher prices per unit of milk paid to farmers\textsuperscript{41}. Additionally, many Ethiopians prefer the taste of traditionally processed milk and dairy products as opposed to pasteurized products\textsuperscript{42}.

While informal market still dominate the dairy industry in Ethiopia, the increase in demand for high-quality and value-added products in urban centers provides opportunities for peri-urban, urban, and even rural small-scale farmers to access and benefit from the market\textsuperscript{36}. Also, quality standards and regulations can be set for milk entering the formal market, providing a safer product for consumers. Prior to 1991, the formal market consisted only of the milk processed through the Dairy Development Enterprise (DDE), a government-controlled agency\textsuperscript{39}. Today, there are nearly a dozen private milk processing plants in and around Addis Ababa. The privately-owned Agro-Sebata Industry processes 28,000 liters of milk per day accounting for 50\% of the total milk processed. The remaining private plants process anywhere from 1,000 to 3,000 liters of milk per day. While DDE and Agro-Sebata account for a large majority of the milk produced, the operation of several smaller private milk processing plants provides a limited but growing amount of competition\textsuperscript{42}.

In addition to the development of private processing plants, milk marketing co-operatives have been established to facilitate a market-oriented dairy industry. According to the FCA’s 2008 annual report, there are 23,167 primary co-operatives throughout the nation that provide a total of 4.6 million individual members with an outlet for surplus milk. To further strengthen bargaining power of the primary co-operatives, 143 co-operative unions have been established\textsuperscript{45}. The co-operative system provides farmers with a consistent outlet for their milk as well many other benefits that help improve production and keep operating costs low. Well managed milk co-operatives are able to minimize transaction costs, reduce price fluctuations
over the seasons, increase production efficiency, improve incomes and employment opportunities, reduce the waste of milk due to poor handling procedures and lack of processing facilities, and provide training on dairy management and proper milk hygiene procedures\textsuperscript{46}.

Addis Ababa and the surrounding areas have many established dairy co-operatives and hold a large potential for further development. These include Addis Ababa, Holetta, Sululta, Selale, Sheno, Debre Birhan, Nazareth, Arsi, and Debre Zeit.\textsuperscript{9} Debre Zeit and Selale are examples of milk shed areas that have established strong dairy co-operative unions and have seen success and growth in the dairy industry\textsuperscript{46-48}. Asela Town also holds potential for dairy development and is making progress in promoting a market oriented system\textsuperscript{48}.

Ada’s Dairy Co-operative, established in Debre Zeit, is an example of a well-managed dairy co-operative. In 2007, the co-operative consisted of 850 members owning 3,000 dairy cows and 10 collection centers who collected on average 8,000 liters of milk per day. The annual milk collection grew from 288,000 liters in 2000 to 2.6 million liters in 2005. Most of the milk collected is sold to DDE, where it is pasteurized and sold in Addis Ababa. The rest of the milk is sold directly to hotels and restaurants in Addis Ababa and other urban centers further away. Ada’s Dairy Co-operative also has their own processing unit to process surplus milk into butter, yogurt, and cottage cheese if needed. Members of the co-operative receive grass hay, concentrate feeds, veterinary drugs and services, and AI services at an affordable price. Members owning two improved milking cows make an average income of 200 USD per month which is considerably higher than the national average per capita GDP. The co-operative is continuing to grow as more rural farmers are joining for market access\textsuperscript{46}.

Selale is located to the west of Addis Ababa, in the North Shoa Zone of the Oromia Regional State where dairy is a predominant farming activity. In 2001, the Selale Dairy Co-operative Union was established to supply feed and veterinary services at a reduced price. The
co-operative now collects and markets milk in 5 woredas; Mulona Sululta, Wuchalena and Jido, Girar Jarso, Yaya Gulele and Debre Libanos, and Degem. A strong market link has been established between the co-operative and Addis Ababa. The head co-operative office is located in Chancho, a town 45km west of Addis Ababa and collection centers have been established between 20 and 140km to the northwest of Addis Ababa. The Selale Dairy Co-operative Union currently has 1,700 active members and receives 8,000 to 10,000 liters of milk daily. The milk is carried mostly in plastic containers by foot or donkey by the dairy producers to the collection centers. Thirty collection centers have been established, all located near an asphalt road. Quality tests using a lactometer and alcohol test are performed at the collection center, at the head co-operative, and by the processors. Only one collection center owns a refrigerator, but it was not functional at the time of this survey. Most of the milk collected is sold to private processors in Addis Ababa, but fresh milk is also sold to shops and super markets in the city. Collection centers themselves often serve as shops for selling fresh milk and processed products. The co-operative plans to establish its own processing plant, strengthen cooling mechanisms, reduce transportation costs, expand the number of milk collection centers to include more members, and improve the capacity for quality testing.

Asela is located at the foot of Mount Chilalo on the eastern side and has productive climate and soil conditions. There are approximately 1,700 dairy cows in Asela that have the estimated potential to produce 30,000 liters of milk per day. Considering this potential, the Asela Dairy Producers Co-operative was established. Currently the co-operative has 89 members, however only 28 members supply milk due to the co-operatives low capacity to store, process, and market milk. The members provide the co-operative with 168 liters per day. Only 20% of the milk is sold fresh while the remaining is processed to products with longer shelf lives such as yogurt, cream, and butter. While there is potential for a successful market oriented
system in Asela, more collection centers need to be developed as well as cooling and processing facilities. An improved supply chain and market link with the nearby town of Adama would also be conducive to improving Asela’s dairy industry\textsuperscript{48}.

2.8 Quality Control Measures

As previously mentioned, milk can play a role in transmitting zoonotic and foodborne diseases if it is not handled under the proper hygienic conditions. If the milk is of low quality and contains bacteria of public health significance, the nutritional value is undermined. Ethiopia, as a developing country, faces many challenges in producing a quality product that is safe for consumption\textsuperscript{20}. Because there is little access to refrigeration throughout the dairy system and less than 1% of milk produced is pasteurized, focus needs to be placed on measures promoting clean milk production and minimizing the time between milking and consumption or transport to a point of sale or processing\textsuperscript{20,34}.

In the United States, several preharvest measures are taken during the milking process to reduce the risk of contaminating milk. Many farms provide training to personnel in proper milking procedures as well as the rationale behind the procedures. The use of gloves while milking is recommended to prevent the passage of pathogens from the milker to the cow. The gloves must be disinfected between cows. Cows presenting with clinical mastitis should be milked at the end and with separate equipment\textsuperscript{51}. Pre-milking teat disinfection is important in reducing environmental bacteria on the teat surface and the bacterial counts in the milk\textsuperscript{52}. Fore-stripping improves the quality of milk by allowing the farmer to observe any abnormalities in the milk and removing the initial milk that is high in somatic cells. Drying the teats prior to milking may be the most important step in removing bacteria from the teat surface and in stimulation\textsuperscript{53}. After milking, the teats should be cleaned with a disinfectant to kill mastitis.
causing pathogens before they enter the teat canal. This practice is the most effective in preventing the spread of contagious mastitis\textsuperscript{54}.

Although many of these preharvest practices seem straightforward and easily implemented, there are additional factors to take into consideration in a developing country such as Ethiopia. There is limited data on hygienic practices throughout the dairy production system in Ethiopia and standard milking protocols do not exist. A recent study showed many farmers do not disinfect teats prior to milking. The study also showed a trend of farmers either not using a towel at all for disinfection or using a collective towel for two or more cows. This practice can clearly lead to the spread of contagious pathogens. The majority of farmers in the study did wash their hands prior to milking. The study did not mention the use of other hygienic practices such as fore-stripping, drying, and post-milking disinfection\textsuperscript{9}.

Many collection centers, co-operatives, 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. If milk contains more than 0.21\% acid it will form curds when combined with alcohol\textsuperscript{9,49}. The milk is rejected if it fails either of these tests and is often processed into yogurt and butter within the co-operatives or by the dairy producer\textsuperscript{49}. Unfortunately, neither of these tests can determine the presence of bacterial pathogens of public health significance\textsuperscript{9}. As the dairy industry grows throughout Ethiopia, it is essential to develop standards and procedures for determining the quality of milk and dairy products.

The government is currently looking into developing a quality-based payment system to encourage smallholder producers, co-operatives, and processors to produce high-quality milk
that is safe for the consumer. In order for a quality-based payment system to be successful, smallholder dairy farmers and co-operatives need to have access to the proper equipment to produce quality milk and there must be an obvious return on their investment in such equipment\textsuperscript{9}.

2.9 Public Health Impact

Malnutrition throughout the developing world is considered the “silent emergency” by the United Nations Children’s Fund (UNICEF). Developing fetuses, children under the age of three, and women before and during pregnancy and while breastfeeding are affected most critically. Over half of the 12 million deaths of children under five in developing countries are caused directly or indirectly by malnutrition\textsuperscript{55}. In addition to high death rates, the “silent emergency” impairs mental and cognitive development in children, makes individuals more susceptible to illness, and can lead to lasting mental and physical disabilities\textsuperscript{55,56}. In Ethiopia, 34.6 million people are undernourished, accounting for 44% of the population, and 47% of children are at or below the stunting weight\textsuperscript{57}.

Because malnutrition is caused not only by inadequate caloric intake (lack of quantity), it is important to focus on integrating animal source foods into the diets of people throughout the developing world in order to improve diet quality. The poor in developing countries often have diets based mainly on cereals and suffer from micronutrient deficiencies. Animal source foods provide proteins containing essential amino acids, energy, and micronutrients that are imperative in decreasing malnutrition. Milk specifically can provide nutritional elements that have been shown to improve growth in children\textsuperscript{56}.

Milk is a highly nutritious product that plays a significant role in maintaining health throughout the world and is especially important in the diets of children and the elderly. While efficient milk production can improve economic development and livelihoods of smallholder
farmers, it can also serve as a means to improve nutrition, especially among children. Milk contains essential nutrients for normal growth and development such as calcium, phosphorus, riboflavin, vitamin B<sub>12</sub>, protein, potassium, zinc, magnesium, and vitamin A. The USDA recommends between two and three servings of dairy products per day in the 2005 Dietary Guidelines for Americans based on the role milk plays in improving physical growth and its additional benefits. Studies have consistently shown that the consumption of milk and dairy products is associated with the prevention of obesity, hypertension, diabetes, colon cancer, and osteoporosis. These are all diseases that developing countries are beginning to face as they become more industrialized and life expectancy increases.

The economic and nutritional value of milk and dairy products in developing countries is evident. However, as the industry grows and becomes more market oriented, focus needs to be placed on the potential risks associated with dairy production and consumption. In developed countries, up to 30% of the population is affected by a foodborne illness per year causing great strain on public health and the economy. The American food supply system is among the safest in the world, but there are still an estimated 76 million cases of foodborne illness a year causing 5,000 deaths and 325,000 hospitalizations. The major pathogens alone are responsible for $35 billion a year in medical costs and loss of productivity. Information on the impact of foodborne illness in developing countries is limited due to lack of reporting systems and poor health care infrastructure. Even so, the burden of foodborne illness in developing regions is estimated to be great based on the high number of diarrheal diseases. In 2005, 1.8 billion deaths of children under 5 worldwide were attributed to diarrheal disease, and a large portion was due to contaminated food and drinking water. Of the 1.8 billion deaths, 78% (1.46 billion) occurred in Africa and Southeast Asia. Diarrheal diseases in developing countries are a great public health concern due not only to their direct cause in illness and morbidity, but also their role in
malnutrition in infants and young children\textsuperscript{61}. If a child is sick with a diarrheal disease, the inability to absorb nutrients undermines the nutritional benefits of a diet sufficient in quantity and quality and can exacerbate malnutrition\textsuperscript{64}.

Milk and dairy products are a potential source of transmission for many foodborne pathogens due to a neutral pH and rich nutrient composition\textsuperscript{65}. Milkborne outbreaks in the U.S. and other industrialized countries have been drastically reduced over the years due to the great amount of focus that is placed on quality control, to include the widespread use of pasteurization, the guidelines set forth in the Grade “A” PMO, and HAACP procedures. In 1938, milkborne outbreaks accounted for 25\% of all outbreaks in the U.S. due to contaminated food and water. Currently, milkborne outbreaks account for less than 1\% of all foodborne outbreaks in the United States\textsuperscript{6}. The majority of documented milkborne outbreaks have been the result of unpasteurized dairy products. Between 2000 and 2006 in the United States, 40 outbreaks were traced back to raw milk compared to only 4 from pasteurized milk\textsuperscript{66}. Throughout the developing world, over 80\% of the milk consumed is unregulated, and in Ethiopia less than 1\% of the milk consumed is pasteurized\textsuperscript{20,34}. Again, there is limited information on the impact of milkborne disease in these regions, but based on the large amount of unregulated milk consumed and the risks of consuming unpasteurized dairy products, the impact is likely to be great.

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)\textsuperscript{65}. As the dairy industry in developing countries moves towards a more market-oriented system, food safety becomes exceedingly important. When there is contamination with mass distribution, outbreaks affect more people
and cause a greater economic impact. Focus needs to be placed on food safety standards and procedures for both preharvest and postharvest activities.\textsuperscript{65}

To prevent preharvest contamination, focus is placed on animal health. Improving milk hygiene and pasteurization have proven to be effective in preventing postharvest contamination.\textsuperscript{65} The dairy farm environment plays a large role in post harvest contamination because many foodborne pathogens inhabit the ruminant intestinal tract and are released into the environment though fecal matter.\textsuperscript{67} Without proper handling of the manure, these pathogens can infect other animals or humans through direct contact or through the contamination of food products such as milk.\textsuperscript{68} \textit{Staphylococcus aureus} and \textit{Salmonella enterica} are two pathogens of public health significance that contaminate milk through both preharvest and postharvest activities.

2.9.1 \textit{Staphylococcus aureus}

\textit{S. aureus} is responsible for nearly all Staphylococcal food poisoning (SFP) cases throughout the world which account for a large portion of gastroenteritis in general. The true morbidity of SFP is unknown, however the World Health Organization (WHO) estimates that in the U.S. cases exceed 185,000 annually, are responsible for 1,750 hospitalizations, and cost $1.5 billion per year.\textsuperscript{69} In the European Union, SFP accounts for 4.1% of all reported outbreaks.\textsuperscript{70} The case fatality rate among the general public is 0.03% but can be as high as 4.4% among susceptible populations - the elderly, children, and individuals with weakened immune systems.\textsuperscript{71,72} In Ethiopia, children under 14 make up over 40% of the population, and although limited prevalence information is available, a significant portion of the country is living with HIV/AIDS.\textsuperscript{37,73} This, combined with poor food safety practices, suggests that the impact here is as great as or greater than in developed areas, although there is limited data.
SFP is characterized by the rapid onset of abdominal cramps, nausea, vomiting, and diarrhea. The symptoms typically begin within 30 minutes to 8 hours and are self-limiting within 24-48 hours, but in rare cases they can lead to hospitalization or death\(^\text{74}\). SFP is a toxin-mediated illness referred to as a foodborne intoxication as opposed to infection because the associated organism, \textit{S. aureus}, does not require growth in the host to produce gastroenteritis\(^\text{71}\). When food products are contaminated with \textit{S. aureus}, certain enterotoxigenic strains can produce one or more staphylococcal enterotoxins (SEs), that when ingested, cause SFP\(^\text{69}\). Other Staphylococcal species are capable of producing SEs but \textit{S. aureus} produces a larger amount than most species and therefore poses a greater public health risk\(^\text{71}\).

Seven different SEs have been shown to elicit emetic responses; SEA, SEB, SEC\(_{1,2,3}\), SED, SEE, SEG, and SEI. Other staphylococcal-like (SEI) proteins have been identified but either do not elicit emetic activity in a primate model (SE/I and SE/Q) or have not been tested (SE/J, SE/K, SE/M, SE/P, SE/U, SE/U\(_2\), SE/V)\(^\text{75}\). SEs are strong gastrointestinal exotoxins that are produced by \textit{S. aureus} throughout replication. All SEs, except SEA, are produced at higher rates during the late exponential growth of \textit{S.aureus} \(^\text{69}\). This puts food products that stay at room temperature for extended periods of times at higher risk for being contaminated with SEs. Additionally, SEs are resistant to heat, low pH, and proteolytic enzymes, making approaches to mitigate SFP difficult\(^\text{69,71}\). Even after boiling and pasteurization, SEs retain their biological activity\(^\text{69}\). The infectious dose for some SEs is as low as 100-200ng, as shown in an outbreak of SFP from chocolate milk contaminated with SEA\(^\text{76}\). A study isolating \textit{S. aureus} from dairy products originating from different animal species throughout Italy found 67% of the isolates to be positive for one or more enterotoxigenic genes. The study included SEI proteins as well. The majority of the isolates were from cow milk and genes \textit{sea} and \textit{sed} were the most common\(^\text{77}\).
An additional study in Korea found 31.8% of *S. aureus* strains from bovine mastitic milk to be enterotoxigenic with SEA, SEB, and/or SEC the most commonly found\textsuperscript{78}.

*S. aureus*, the organism responsible for the synthesis of SEs, is a gram-positive organism that is ubiquitous throughout the environment, but the most common reservoirs are humans and animals\textsuperscript{69}. Humans and animals are also the primary sources for food contamination\textsuperscript{71}. Studies show that 20-30% of the general population are colonized with *S. aureus*\textsuperscript{69}. Adapting information from the NHANES survey, 31.6% of the U.S. population is colonized with *S. aureus* and 0.84% with methicillin resistant *S. aureus* (MRSA)\textsuperscript{79}. Further increasing the public health significance, one-third to one-half of the isolates are estimated to be enterotoxigenic\textsuperscript{80}. Colonized food handlers are the most common source of dissemination. Most cases of SFP are traced back to human carriers who contaminate the food during processing or preparation\textsuperscript{71}. Foods that require extensive handling and are left at room temperature for extended periods of time are usually implicated in SFP outbreaks\textsuperscript{69}.

Milk and milk products are among the most common foods associated with SFP outbreaks and cases; however, the proportion of SFP attributable to milk varies between different countries with different eating and cultural habits and milk production systems\textsuperscript{69}. In the United Kingdom between 1969 and 1990, 8% of outbreaks were due to milk products\textsuperscript{81}, whereas in France between 1999 and 2000, milk products were responsible for 32% of SFP cases\textsuperscript{82}. In France, the consumption of unpasteurized milk products is much higher, which may explain the difference. In the United States, where a very small percentage of unpasteurized dairy products are consumed, less than 1% of outbreaks are due to contaminated milk products\textsuperscript{6,74}.

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,
*S. 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 giving enterotoxigenic *S. aureus* ample time to proliferate and produce an abundance of SEs. As mentioned earlier, SEs retain their toxicity after both boiling and pasteurization. Postharvest food safety practices, including milking hygiene and refrigeration, are essential to reduce the incidence of SFP through the consumption of contaminated milk.

While humans are a strong determinate in the transmission of *S. aureus* to food products, animals play an important role as well. *S. aureus* is the most common cause of subclinical and clinical mastitis throughout the world and the most economically damaging organism in the dairy industry. Mastitis reduces milk yield, profit margins, and the quality of milk and milk products. Studies show that in most countries the prevalence of mastitis is 50% in cows and 25% in quarters. In Ethiopia, studies have found *S. aureus* to be implicated in approximately 40% of mastitic cows. The economic impact of mastitis in developing countries is great. A study on cross-bred dairy cows in Debre Zeit shows that mastitis reduces potential milk yield by 34.5% and incurs a financial loss of 984.64 Eth Birr (78.65USD) per cow per lactation period. In addition to the economic loss *S. aureus* can cause though mastitis, it is also a public health threat when it is excreted into the milk through a mastitic cow. A significant amount of mastitic strains of *S. aureus* are enterotoxigenic. In a study on mastitis in Trinidad, 105 strains of *S. aureus* were isolated from bulk milk. Of these, 42.9% were enterotoxigenic. In Ethiopia, the high prevalence of mastitis caused by *S. aureus*, combined with limited veterinary services, poor milking hygiene and the inability to refrigerate milk prior to consumption, suggests that milk and milk products are an important source of SFP throughout the region.

2.9.2 *Salmonella enterica*
Salmonella species are gram-negative bacteria that are commonly found in the intestinal tract of cold- and warm-blooded animals and are also ubiquitous in the natural environment. They have an optimal growth temperature between 35° and 37°C but can survive at temperatures between 2 and 46°C. Salmonella organisms can be killed by pasteurization temperatures and times and are also sensitive to pH levels lower than 4.5. Nevertheless, Salmonella are resilient and can adapt to extreme environments, as demonstrated by their ability to grow in foods stored at 2 to 4°C which adds to the public health significance. The most important strain in regards to public health is Salmonella enterica. There are over 2,000 subspecies, all of which cause Salmonellosis. Salmonella enterica subspecies typhimurium and enteriditis cause the highest number of foodborne illness worldwide and affect both animals and humans. Salmonella typhimurium is frequently found in food animals and pets, whereas Salmonella enteritidis is prevalent among poultry and eggs.

Salmonella can cause acute or chronic disease in animals, or it can be asymptomatic. In dairy cows, Salmonella infections are often asymptomatic but can also cause salmonellosis consisting of fever and diarrhea. In some cases, the condition can develop into bacteremia or endotoxemia and cause abortions. When dairy cows are asymptomatic, they continue to shed Salmonella in their feces, leading to the potential for transmission via the fecal oral route or through the contamination of food products. According to a national study in the United States looking at 91 dairies from 19 states, 21.1% of dairies had a least one cow shedding Salmonella in their feces. A study conducted on 450 cattle from Addis Ababa, Ethiopia and surrounding areas isolated Salmonella from only 8 cows (1.8%). This is much lower than what is found in the U.S. and may be due to the differences in production systems. The intensive production system of the U.S. allows for more frequent horizontal transmission of Salmonella in cows. Even with the low prevalence found within cattle in Ethiopia, isolates of Salmonella have been recovered from...
milk and milk products, proving there is a need for improved hygiene throughout the dairy production system\textsuperscript{97}.

The same strains that infect dairy cattle can cause salmonellosis in humans as well. Salmonellosis is a foodborne infection, meaning that the bacteria multiply and invade the intestinal tract mucosal cells and produce enterotoxins and cytotoxins that destroy epithelial cells, causing gastroenteritis\textsuperscript{71}. The onset of symptoms in humans starts between 8 and 48 hours and typically last for 2 to 5 days. They consist of abdominal pain, cramps, diarrhea, nausea, and vomiting. Salmonellosis is often self-limiting but can create complications in children and elderly due to loss of fluids. Treatment is usually limited to supportive therapy consisting of fluid and electrolyte replacement. The use of antibiotics to treat Salmonellosis is not recommended because it leads to a longer carrier state where the patient periodically can still shed salmonellae in their feces. In rare cases, infections can become systemic and cause chronic conditions\textsuperscript{93}. In these cases, it is essential to treat the infection with effective antimicrobials\textsuperscript{98}.

Individuals with preexisting physiological, anatomical, and immunological disorders are more likely to develop severe and protracted illness because their immune systems lack the ability to effectively fight invasive salmonellae\textsuperscript{99}. The risk factors for developing Salmonella bacteremia are much higher in developing countries than in developed countries. In Sub-Saharan Africa, the high prevalence of HIV, malaria, malnutrition, and anemia make the population more vulnerable to severe complications regarding Salmonella infection. A 4 year study of invasive bacterial infection in 16,570 children admitted to a Kenyan hospital showed Salmonella to be its second most common cause at 1\%\textsuperscript{100}.

*Salmonella* is one of the most common causes of bacterial foodborne outbreaks throughout the world and has a great economic impact. In the United States, there are an estimated 1.4 million cases of Salmonellosis per year in humans, causing 14,860 hospitalizations
and 400 deaths\textsuperscript{101}. This accounts for 9.7% of total foodborne illnesses and is the cause of 31% of deaths associated with foodborne transmission\textsuperscript{102}. From 1993 to 1998, 77% of all reported outbreaks in the European Union were due to \textit{Salmonella} and 55% of all foodborne transmitted cases were due to \textit{Salmonella} infections\textsuperscript{70}. Information on Salmonellosis prevalence in developing countries is limited due to isolated communities, poor health care infrastructure, and lack of a reporting system\textsuperscript{103}.

In agricultural areas where humans work closely with livestock, there is a large potential for human infection through fecal contamination of animal food products, especially in the dairy sector. In the United States, contracting salmonellosis through milk contaminated on dairy farms is of little concern due to wide scale pasteurization\textsuperscript{95}. In developing countries, however, where a large majority of the milk is not pasteurized, the risk is significantly greater. Ethiopia’s dairy production system consists mostly of small-scale dairy farms that often lack available resources for proper hygiene and are operated by a small number of workers who share all of the chores involved in running the dairy, including manure management. This leads to a high potential for milk to be contaminated with feces. Without access to pasteurization, preharvest food safety within milk production is extremely important in reducing the risk of Salmonellosis in Ethiopia and other developing countries\textsuperscript{65,68}.

\textbf{2.9.3 Antimicrobial Resistance}

In addition to the public health risk caused by Salmonellosis and SFP, certain strains of \textit{Salmonella} and \textit{S. aureus} are antibiotic-resistant due to a variety of factors, including an increase of antibiotics given to food animals. The liberal use of antimicrobial agents at hospitals and treatment centers, as well as their subtherapeutic use in livestock for growth promotion and prophylaxis, greatly contributes to the emergence and persistence of resistant strains\textsuperscript{93,104}. The main concern is that the pool of resistance genes is increased and can be spread through
bacterial plasmids and other mobile genetic elements. The use of antibiotics in animal husbandry often leads to a higher level of resistance against that drug. In pathogens with zoonotic potential, like *Salmonella* and *S. aureus*, antibiotic resistance reduces our ability to treat both humans and animals, increasing their public health significance\textsuperscript{104}.

The prevalence of antimicrobial-resistant *Salmonella* has increased over the past decades. Between 1980 and 2001 *S. typhimurium*, the most common serotype isolated from humans in the United States, increased resistance rates to one or more of nine antimicrobial agents from 13\% to 51\%\textsuperscript{105}. In general, there has been an increase in resistance levels in some, but not all serovars linked to animal husbandry\textsuperscript{104}. When first administered to humans, fluorquinolones, a category of drugs often given to treat invasive Salmonellosis, did not cause a significant rise in resistance. However, when subsequently used in livestock, rates of resistant *Salmonella* increased in animals, food, and humans\textsuperscript{106}.

Single-drug resistance in *Salmonella* has been reported since the early 1960s, and it was not until the 1970s that multidrug resistant strains were recovered. In 1979, an *S. typhimurium* clone of phage type 204 was isolated from both bovine and humans. The clone carried resistance against chloramphenicol, streptomycin, sulfonamides, and tetracycline. Other multidrug resistant strains were subsequently identified; today, resistance genes have been integrated into the bacterial chromosome of some variants, making drug resistance essential to the survival of these *Salmonella* strains\textsuperscript{98,104,107,108}. Chromosomal integration of resistance genes means that even if the selective pressure of antibiotic use is removed, the strain will still retain the resistance gene\textsuperscript{98}. Currently, *S. typhimurium* phage type DT04 is the predominant antimicrobial resistant strain\textsuperscript{104}.

*S. typhimurium* phage type DT104 is multiply resistant, geographically widespread, and has been isolated from humans and all food producing animals. DT104’s resistance genes are
integrated into its chromosome. Most of the DT104 isolates are pentaresistant to ampicillin, chloramphenicol, streptomycin, sulphaomides and tetracycline (R-type ACSSuT), but some strains have shown resistance to gentamycin, trimethoprim, and flouroquinolones. Today, DT104 strains are most often found in cattle, which have been shown to be an important reservoir in the transmission of the strain to humans\textsuperscript{104}.

In addition to being difficult to treat, multiply resistant Salmonella serovars are more likely to cause bacteremia, meningitis, or infections of other extra-intestinal organs, especially in the young, elderly, or immunocompromised. During 1996 – 2001, the National Antimicrobial Resistance Monitoring System (NARMS) showed that patients with antimicrobial–resistant nontyphoidal \textit{Salmonella} infection were more likely to have bloodstream infection and to be hospitalized than were patients with pansusceptible infection\textsuperscript{109}. A study in Ethiopia looked at pediatric patients presenting symptoms of diarrhea or fever from two hospitals, one in Addis Ababa and one in Jimma. \textit{Salmonella} was most commonly isolated from the patients with \textit{S. concord} as the predominant serotype. Nearly 70\% of the strains were resistant to trimethoprim–sulphametaxole, ceftriaxone, chloramphenical, and gentamicin and over one–fourth had reduced susceptibility to ciprofloxin. In general, the strains were found to be highly invasive and highly resistant\textsuperscript{110}. The high level of resistance to these antimicrobials is especially concerning because, in Ethiopia, newer classes of antimicrobials such as cephalosporins and quinilone drugs are largely unavailable and expensive\textsuperscript{99}.

\textit{Staphylococci} are naturally susceptible to most antibiotics but are also very prone to the development of resistance genes through mutation and DNA transfer\textsuperscript{111}. Resistance in \textit{S. aureus} developed very quickly after the introduction of penicillin in the 1940s. Today there are strains of \textit{S. aureus} that are resistant to the most commonly used antibiotics\textsuperscript{112}. MRSA is a strain that is
resistant to beta-lactams, including methicillin and often oxacillin, penicillin, and amoxicillin^{113}. MRSA was originally associated with nosocomial infections and still remains a problem in hospitals throughout the world today^{114}. Within the healthcare setting, risk factors include patients with a weakened immune system, recent surgery, and insertion of a catheter among other things. Hospital Associated (HA)-MRSA is commonly associated with surgical wound infections, urinary tract infections, bloodstream infections, and pneumonia^{113}. Antimicrobial resistance in general continues to grow in hospitals. The National Nosocomial Infection Surveillance (NNIS) system has shown an increase in the proportion of *S. aureus* strains isolated from patients in U.S. hospitals that are resistant to methicillin, oxacillin, or nafcillin (60%)^{105}.

Not only is there still a concern with HA-MRSA, but the prevalence of community associated (CA) MRSA is growing. Everyone is at risk for CA-MRSA and can be infected through skin-to-skin contact, cuts and abrasions, contaminated items or surfaces, living in a crowded space, and poor hygiene^{113}. MRSA in livestock animals is another contributing factor in human infections. A study in the Netherlands found a new MRSA strain from an animal reservoir that entered the human population and now is responsible for more than 20% of all MRSA strains in the Netherlands. The study also showed that the strain was more likely to be isolated from pig and cattle farmers^{115}.

As previously mentioned, resistance among *S. aureus* is a public health issue worldwide. A study conducted in the 1980s in Addis Ababa, Ethiopia, sampling hospital and non-hospital populations, isolated *S. aureus* from 32.4% and 21.6% respectively. Over 96% of the hospital strains and 88% of the non-hospital strains showed resistance to at least one antibiotic and 45% of the hospital strains were multiply resistant^{116}. A more recent study in Southwestern Ethiopia determined 8.3% of *S. aureus* isolates from clinical specimens at the Jimma hospital were MRSA and over 90% were resistant to penicillin and ampicillin^{117}. 
Resistant *S. aureus* strains have also been isolated from cattle throughout Ethiopia.

Studies isolating *S. aureus* from bovine mastitic milk show high levels of resistance to ampicillin, penicillin, polymixin B, and streptomycin$^{89,90}$. Considering the large portion of the Ethiopian population that lives in close proximity to their livestock, there is potential for transmission of resistant *S. aureus* from livestock to humans through the consumption of milk.
Materials and Methods

3.1 Description of Study sites

The study area included milk collection centers throughout central Ethiopia. Our samples represent peri-urban and urban dairy farms from Selale, Asela, Debre Zeit, and Akaki participating in the formal milk market. Refer to Figure 1 for a map of the regions. The 16 collection centers sampled were chosen based on location and willingness to cooperate. The first ten collection centers sampled are from Selale (Se 1-10) and are part of the Selale Milk Producers Co-operative Union. The majority of samples are from this region based on the strong relationship the co-operative has with the Netherland Development Group (SNV) and the large amount of milk the area produces. The Selale Co-operative Union consists of 27 collection centers and 30 collection points where approximately 1,700 active members supply between 8,000 and 10,000 liters of milk per day. The collection centers are located as far as 140km from Addis Ababa and the average temperature ranges from below 10°C to 15°C from an elevation of 2300 meters to above 3300 meters. The Asela Dairy Producers Co-operative is a much smaller organization and only three collection centers (As 1-3) were sampled from this area. The co-operative has 89 members however not all members bring milk to collection centers due to a low capacity to store, process, and market the milk. The climate in Asela consists of the Dega agro ecological climate with temperatures ranging between 10°C and 15°C and elevations ranging between 2300 and 2800 meters. The environmental conditions are productive with regards to soil and climate. Debre Zeit is located only 45 km southeast of Addis Ababa and has a climate conducive to dairy production. The area’s 10 collection centers are part of the Ada’a Dairy Co-operative which consists of 813 members and over 3000 cows that collectively bring approximately 8,000 liters of milk per day to the co-operative. Only two collection centers
were sampled due a smaller pool of collection centers and bureaucratic issues that prevented further sampling. From Akaki, we sampled one collection center to represent the total of four to six collection centers in the area. The Akaki Dairy Co-operative consists of 250 members who produce approximately 2,000 liters of milk per day. Akaki is located 30km southeast of Addis Ababa at an elevation of 1850-1950 meters. The climate is subtropical with temperatures ranging 18 - 25°C. Of the total milk produced in the area, 75% is marketed through the formal channels.

3.2 Collection Center Selection

During the two month project 155 total samples were collected representing small-scale farmers who bring their milk to collection centers in Asela (n=33), Selale (n=106), Debre Zeit (n=5) and Akaki (n=10). Selale samples were collected from 10 collection centers on two separate occasions totaling 106 samples. Asela samples were collected from 3 collection centers and one large scale farm for a total of 33 samples. Both Selale and Asela collection centers were visited twice. A total of 10 samples were collected from two Addis Ababa collection centers in Akaki and 5 samples from one collection center in Debra Zeit. When disregarding bulk tank samples and the samples from the individual farm, the milk we sampled from each farmer was pooled from 2.08 cows on average; hence, 260 cows were essentially sampled.

3.3 Sample Collection

The sampling period took place during the rainy season in July and August 2010. The sampling team consisted of three individuals and sometimes a fourth individual to assist with interpreting the questionnaires. Transportation to and from the different sites was arranged through The Netherlands Development Organization (SNV). The arrival time at the collection centers was during either the morning collection time, between 6:30am and 8:30am, or evening
collection time, between 4:00pm and 5:30pm. The owners of the collection centers were informed of the purpose of the study prior to the collection date through the Dairy Co-operative of the region. Upon arrival, the collection team reiterated the purpose of the study to both the owners and the farmers. At each collection center 4-6 samples from individual bulk tanks were taken and one sample was taken from a combined bulk tank. An individual bulk tank consisted of milk from all dairy cows on the individual’s farm whereas the combined bulk milk tanks consisted of several individual farmers’ milk combined. The individual performing sample collection wore latex gloves and took convenient samples from individual farmers by dipping a sterile sample container into the milk jug before it was mixed into the combined bulk tank. Once the milk was combined, one sample was taken in the same manner from the bulk tank. The sterile containers were sealed tight and clearly marked for identification purposes. All samples were placed on ice and transported to the Aklilu Lemma Institute of Pathobiology (ALIPB) laboratory for processing.

3.4 Questionnaire Administration

In addition to collecting samples, questionnaires were administered to each participating farmer and the collection center owner to assess current milking practices and possible risk factors regarding the contamination of milk. Questionnaires were administered to the individual who brought the milk to the collection center immediately after the samples were taken. The questions were originally written in English and translated into the local language when administered. The answers were then translated to English and entered into the original form. To facilitate further analysis, the information was entered into an excel spreadsheet. Refer to appendix A for the questionnaire and Appendix B for an explanation of terms used for further analysis.
3.5 Laboratory Procedures performed at Akilu Lemma Institute of Pathobiology (ALIPB)

Processing of samples began immediately upon returning to the lab. After shaking the sample containers thoroughly, one milliliter of each sample was saved in an appropriately marked cryopreservation vial and placed in the freezer at -20°C for further processing at The Ohio State University (OSU).

3.5.1 *S. aureus* isolation/identification

For the isolation of *S. aureus* standard laboratory procedures were followed. After thoroughly shaking the sample container, one loopful of milk was plated onto mannitol salt agar (Becton, Dickenson and Company, Sparks, MD) and incubated for 24 hours at 37°C. An isolated colony from all positive mannitol salt plates was struck onto Luria Bertani (LB, Becton, Dickenson and Company, Sparks, MD) agar plates for use in two biochemical tests, catalase (H₂O₂) and coagulase (BBL Coagulase Plasma, Rabbit with EDTA, Becton, Dickinson and Company, Sparks, MD). Positive reactions for the presence of the catalase enzyme were evident by immediate effervescence. Only catalase positive isolates were tested for coagulase. After mixing a loopful of the bacteria into the coagulase tubes, they were vortexed and incubated at 37°C for 24 hours. Positive reactions for the coagulase test were determined by observing clotting in the tube. Isolates that were positive for both biochemical tests were streaked onto LB slants to keep at ALIPB as well as cryopreservation vial LB slants for transport to OSU.

3.5.2 *Salmonella* isolation/identification

Isolation and identification of *Salmonella* was done following conventional methods as described previously (Patchanee *et al.*, 2010) with some modifications. Briefly, 25 ml of milk samples was preenriched in equal volume (25 ml) of lactose broth (Becton Dickinson and Company, Sparks, MD) with a 1:1 v/v ratio and incubated at 37°C overnight. For the first two
weeks of sampling two separate ratios of milk to lactose broth were used (1:1 and 1:9) to
determine which ratio gave better results. After the testing phase, the 1:1 ratio was chosen
based on our results and previous research isolating *Salmonella* from water. About 100 μl of
the pre-enriched suspension was transferred into 9.9 ml of Rappaport–Vassiliadis broth (Becton
Dickinson and Company, Sparks, MD) and incubated at 42°C for 24 h. A loopful of the selective
enrichment was plated onto XLT4 (Xylose Lysine Tergitol 4; Becton Dickinson and Company,
Sparks, MD) plates and incubated at 37°C for 24 h. Up to 10 presumptive *Salmonella* colonies (5
per XLT4 plate) were selected and tested for biochemical and serological reactions. All isolates
were further subjected to biochemical testing using triple sugar iron agar (Becton Dickinson and
Company, Sparks, MD) and urea agar slants (Becton Dickinson and Company, Sparks, MD) for
confirmation. Isolated colonies that were positive for both biochemical tests were streaked
onto LB slants to keep at ALIPB and cryopreservation vials LB slants for transport to OSU.

3.6 Laboratory Procedures performed at the Ohio State University (OSU):

3.6.1 Cryopreservation of isolates

Upon arrival back to OSU, *S. aureus* isolates were refreshed on tryptic soy agar (TSA, Becton
Dickinson and Company, Sparks, MD) plates from the cryopreservation vial LB slants. A 3-phase
streak was used for isolation of colonies and plates were incubated at 37°C for 24 hours.
Isolated colonies were transferred to brain heart infusion (BHI, Becton Dickinson and Company,
Sparks, MD) slants and cryopreserved. The cryopreservation tubes containing 1 ml of milk were
placed in a -80°C freezer for storage for future analyses.

Sterile inoculating loops were used to pick an isolated colony from the TSA plate and
placed into 3ml of Tryptic Soy Broth (TSB, Becton Dickinson and Company, Sparks, MD) in a
16x150 mm sterile glass culture tube. The tubes were then placed in a 37°C shaking water bath
set at 250 rpm for 4 hours making sure the water level covers the broth in the tube. The growth
of bacteria was verified by a cloudy appearance and 1.5 ml of broth and bacteria were transferred into a properly labeled sterile cryovial containing 150 µl (10%) of sterile dimethyl sulfoxide (DMSO, Fisher Scientific, Fair Lawn, NJ). The cryovials were then placed in a -80°C freezer for storage.

3.6.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates was performed using Kirby-Bauer disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI). The test was performed on all S. aureus isolates recovered and quality control strains Escherichia coli ATCC 25922, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, and Pseudomonas aeruginosa ATCC 27853. All isolates used were first refreshed from the LB slants to LB plates under sterile conditions and incubated at 37°C for 24 hours. Five well isolated colonies were taken from the refreshed TSA plates with a sterile cotton swab and transferred to a tube containing 4-5ml of sterile saline solution (0.9%). Inoculums were standardized to 0.5 McFarland using a spectrophotometer. Within 15 minutes, the saline solution was plated on to Müller-Hinton (MH, Becton Dickinson and Company, Sparks, MD) agar plates with sterile cotton swabs. The dry surface of a MH agar plate was inoculated by streaking the swab over the entire sterile agar surface. The streaking procedure was repeated two more times, and the plate was rotated approximately 60° each time to ensure an even distribution of inoculum. Using a sensi-disc designer dispenser, diffusion disks (Becton Dickinson and Company, Sparks, MD) for 12 separate antibiotics were distributed evenly on the MH plate so that they were no closer than 24 mm from center to center. Plates were then incubated at 37°C for 16-18 hours. The antibiotics tested were, Ampicillin (Am; 10 µg), Erythromycin (E; 15 µg), Streptomycin (S; 10 µg), Oxacillin (OX; 1 µg), Tetracycline (Te; 30 µg), Amoxicillin/Clavulanic acid (AmC; 30 µg), Trimethoprim/sulfamethoxazole (SXT; 1.25 µg/23.75 µg), Cetriaxone (CRO; 30 µg), Ciprofloxacin
(CIP; 5 μg), Vancomycin (Va; 30 μg), Penicillin (P; 10 U), and Gentamicin (GM; 10 μg). Zones of inhibition were measured with a ruler to the nearest whole millimeter by at least two individuals and given the appropriate status; Susceptible (S), Intermediate (I), or Resistance (R).

3.6.3 DNA extraction

Genomic DNA was isolated using DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA) with a pretreatment enzymatic lysis buffer as outlined in the instruction manual. Briefly, the lysis buffer contained a mixture of 20 mM pH 8.0 Tris-HCl (Fisher BioReagents, Fairlawn, NJ), 2 mM sodium EDTA (Gibco, Grand Island, NY), 1% Triton X-100 (Sigma, St. Louis, MO), and 20 mg/ml lysozyme (Lysozyme Ultrapure, USB Corporation, Cleveland, OH). Molecular Biology Grade Water (Eppendorf, Hamburg, Germany) was used for the elution step rather than Buffer AE as described in the instruction manual. The resulting elute was stored at 4°C for further genotypic analyses.

3.6.4 PCR for nuc, mecA, enterotoxin genes sea, seb, sec-1, sed, and see and tst

The chromosomal genes encoding for nuc, mecA, sea, seb, sec-1, sed, see, and tst were amplified using previously described methods. The primers used are shown in Table 1. Three separate multiplexes were run. PCR reactions were performed using illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences, Piscataway, NJ). A final reaction volume of 25 µl with 1 µl of template DNA was used. Amplification for the nuc and mecA gene was performed using a PTC-100 Programmable Thermal Controller (MJ Research Inc, Watertown, MA) under the following conditions: initial denaturation at 95°C for 10 min followed by 45 cycles of 95°C for 1 min, 54°C for 1 min, 72°C for 1 min, and a final extension step of 72°C for 1 min. The MRSA ATCC 43300 strain was used as a positive control. Amplification for staphylococcal enterotoxin genes was completed in two separate multiplex reactions (sea, sec-1, tst and see, sed, seb)
under the following conditions: initial denaturation at 95ºC for 10 min followed by 45 cycles of 94ºC for 2 min, 55ºC for 2 min, 72ºC for 1 min, and a final extension step of 72ºC for 7 min. ATCC strains 14458, 19095, 23235, 13565, and 27664 were used as positive controls.

One micro liter of 5X loading dye (Gel Loading Dye, Teknova, Hollister, CA) was added to each PCR reaction. The samples were filtered through a 1% Ultrapure Agarose gel (SeaKem LE Agarose, Lonza, Rockland, ME) containing ethidium bromide (10% of the total volume) in 0.5X TBE buffer (Ultrapure DNA Typing Grade 5X TBE Buffer, 5Prime, Gaithersburg, MD). The amplicons were visualized using Gel Doc 2000 (Bio-Rad Laboratories, Hercules, CA) and analyzed using Quantity One (Bio-Rad Laboratories, Hercules, CA). The size of the amplicons was measured using a 100 bp molecular marker (exACTGene 100bp DNA Ladder, Fisher Scientific, Fairlawn, NJ).

3.6.5 Pulsed Field Gel Electrophoresis (PFGE) genotyping:

DNA fingerprinting of S. aureus isolates (n = 21) was conducted using PFGE. PFGE was performed according to the standardized laboratory protocol recommended by the Canadian Committee for the Standardization of Molecular Methods. The PulseNet “universal” standard strain Salmonella enterica serovar Braenderup H9812 was used as a reference marker. Gel images were then transferred to Bionumerics software version 4.61 (Applied Maths NV) for cluster analysis. Cluster analysis was performed using the unweighted pair group method with arithmetic averages with 2.0% band position tolerances and 1.5% optimization values. Similarity coefficients were obtained within Bionumerics by calculating Dice coefficients. PFGE banding patterns with a similarity index >88.9% were grouped within the same genotypic cluster.
3.7 Statistical Analysis

All analyses were performed using JMP Pro 9 (SAS Inc, Cary, NC). Fisher’s exact test and the Wilcoxon rank sum test were used to determine if there was a statistically significant difference between questionnaire results in peri-urban and urban farms at a p<0.10. Stepwise model selection was used to identify predictors of recovering *S. aureus* from milk. Independent variables consisted of continuous and categorical data collected from the questionnaires. Simple logistic regression was used to determine the unadjusted odds ratio of each variable with regards to *S. aureus* recovery. Associations that were significant at p<0.10 in univariate models were considered for further multivariate regression analysis. Backward selection at p<0.10 was used to determine the final model.

3.8 Genotyping Data Analysis

The presence or absence of bands was determined initially by visual analysis and macrorestriction banding patterns were compared. Clustering of the fingerprints was conducted by the Dice Coefficient Similarity Index. Isolates were clustered according to the UPGMA method using Bionumerics software, version 5.1 (Applied Math inc. Belgium).
Results

4.1 Laboratory Results

4.1.1 S. aureus Isolation

A total of 119 individual farmer samples were collected and S. aureus was recovered from 26% of them. The proportion of S. aureus positive individual farmer samples ranged from 12.5% to 40% across the different regions. Prevalence of S. aureus was higher in the Selale region (28.2%) than in Asela (19%) and Akaki (12.5%). Debre Zeit showed the highest prevalence at 40%. When the regions were combined into peri-urban (26.4%) and urban (23.1%) categories, the prevalence rates were closer (Table 2). The difference in proportions of S. aureus isolated from the different geographical locations, whether categorized by region or peri-urban and urban, was insignificant using ordinary logistic regression. When categorized by the four different regions the p-value was 0.4459 and when categorized into peri-urban or urban farms the p-value was 0.9067.

Twenty-nine combined bulk tank samples were taken, 16 on the first sampling rotation and 13 on the second, and over half of the samples were positive for S. aureus. The proportion of S. aureus positive samples from combined bulk tanks among the different regions ranged from 0% to 60% (Table 3).

4.1.2 Antimicrobial susceptibility

Overall, 87% of 31 S. aureus isolates from individual farmers were resistant to at least one antimicrobial agent. Penicillin, ampicillin, and tetracycline were the only three agents the isolates were resistant to with the frequency of penicillin resistance (74.2%) much higher than both ampicillin (3.2%) and tetracycline (32.3%). Only 16% of isolates were pansusceptible. No
isolates were resistant to oxacillin which is consistent with the absence of the \textit{mecA} gene in all isolates. (Table 4) The most common resistance pattern was Pn accounting for 54.8\% of isolates followed by TePn at 22.6\% and Te and AmPn both at 3.2\%. Approximately one fourth of the isolates were resistant to more than one antimicrobial agent (Table 5).

Isolates from the combined bulk tanks showed similar trends in antimicrobial resistance with 80\% of the isolates showing resistance to one or more antimicrobial agent. Penicillin resistance was most common (87\%) and resistance was also found to ampicillin (13.3\%) and tetracycline (40\%). One isolate from a Selale combined bulk tank was resistant to Streptomycin (6.7\%). Two isolates (13.3\%) were pansusceptible and almost 47\% of the combined bulk tank isolates were resistant to more than one antimicrobial agent.

4.1.3 \textit{Staphylococcal Enterotoxin (SE) genes}

Overall, 29\% of isolates from individual farmers contained one or more SE genes. The SE gene with the highest prevalence was \textit{sea} at nearly 23\% followed by \textit{sec-1} (12.9\%), \textit{sed} (9.7\%) and \textit{seb} (3.2\%). The \textit{see} and \textit{tst} genes were also tested for though no isolates contained these genes. Several isolates (16\%) contained more than one SE gene (Table 6). Again, there were differences in SE gene levels between the different regions but the sample size is not large enough to determine if the differences are statistically significant.

4.1.4 \textit{Pulsed Field Gel Electrophoresis (PFGE)}

PFGE analysis identified four separate clonal types that were clustered geographically and also several sporadic isolates. Cluster A isolates were 89.8\% clonally related and contained only Selale isolates from collection centers 8 and 6 as well as the Bulk Tank samples from 8, 3, and 5. The dates ranged from 7/23/2010 to 7/29/2010. The isolates from collection center 8, farmers 3 and 4 were 100\% identical based on the PFGE banding patterns and genotypic and
phenotypic characteristics. Additionally, isolates from collection center 8, farmers 1 and 2 and
collection center 6, farmer 4 were 100% identical based on the PFGE banding patterns and
genotypic and phenotypic characteristics. The isolate from collection center 6 was taken on a
separate day than the isolates from collection center 8. All isolates in the cluster had the same
resistance pattern and SE gene pattern.

Cluster B consists of three isolates from Selale collection centers 5, 7, and 8 that were
91.8% clonally related. Two of the isolates have the SE gene sea, are 96.8% clonally related, and
are from two separate collection centers.

Isolates in Cluster C were 88.9% clonal. Both isolates are from the one collection center
in Akaki that was sampled. One was from an individual farmer and the other was from the
combined bulk tank. The isolates were phenotypically different in that the resistance pattern
for the bulk tank isolate was AmPn and the individual farmer isolate was pansusceptible.

Cluster D is 88.9% clonally related and consists of two isolates from Asela collection
centers 2 and 4. The isolates were collected on the same day and differ phenotypically. The
isolate from collection center 2 has a resistance pattern of Pn and contains SE gene sed. The
other isolate in the cluster has a resistance pattern of TePn and contains SE genes sea, sec-1,
and sed.

There are also several sporadic isolates. These include 3 isolates from Selale collection
centers, one from a Debre Zeit collection center, and two from the Asela collection center. The
two from Asela were collected 26 days apart but both were taken from the combined bulk tank
(Figure 2).
4.2 Questionnaire Results

4.2.1 Collection center

On average, the collection centers surveyed received 683L of milk a day from an average of 82 farmers. All collection centers administered a lactometer test and 87.5% used an alcohol test to determine whether or not the milk would be accepted. In general, if the milk did not pass the standards it was either returned to the farmer to process at home or kept at the collection center to be processed into cheese or butter. Once the milk arrived at the collection center it was stored in either plastic or aluminum containers until the co-operative truck arrived to transport for processing and/or marketing. The majority of collection centers use both aluminum and plastic containers (62.5%) and the remaining use only plastic (18.8%) or only aluminum (18.8%) containers. Approximately 87% of the collection centers surveyed wash the containers twice a day while the rest only wash the containers once a day. A little more than 78% of the collection centers had access to hot water and tap was the source for the majority of the centers (75%). The remaining four centers used either well or river water. All 16 collection centers used detergent to clean the containers. On average, milk was kept at the collection centers for 44.5 minutes (Table 7). After milk arrived at the collection center, it was either processed at the center, taken to the co-operative to be processed, sold as raw milk from the collection center or transported to be pasteurized and then marketed. Most of the milk in Selale and Akaki was taken to Addis Ababa to be pasteurized as opposed to Asela where most of the milk was either processed locally or sold raw. The one collection center surveyed in Debre Zeit either processed the milk there, sold the milk raw, or sent it to the co-operative to be processed. Only two collection centers offered incentives for quality milk which consisted of small awards such as books or an aluminum milk container. The majority of the collection
centers offered advice to members on how to produce quality milk and some provided feed at an affordable price, training, or helped with vaccine administration.

4.2.2 Individual Farmers

On average, the individual farmers surveyed owned 3.2 dairy cows and milked 2.1 cows. Of the cows owned, cross bred cows were the most common averaging 1.8 per farm and the remaining were either local breeds (0.6) or exotic breeds (0.8). The majority of farmers kept their cows in a tie stall (45.5%) or a free stall (20.5%). Just over 16% kept their cows in a pasture and the rest consisted of a combination of the three (17.9%). In regards to maintaining the health of the cattle, a little over one third of farmers have new cows physically or medically examined prior to entering the herd. Almost half seek treatment for their cows when there are visible symptoms and less than one third have their cows examined more than once a year. Just over 20% of farmers have never had their cows examined. Most farmers said their cows are vaccinated at least once a year through the government and nearly 78% use antibiotics to treat their cows. On average each farm consists of 2.6 workers and the majority of workers milking the cows participate in other activities around the farm which can include manure management (80%). Only 26.8% of farmers said they kept records. The types of records kept consisted of mostly production but some kept medical records as well. All farmers surveyed milk their cows and participated in a variety of preharvest food safety activities. Nearly all farmers clean the teats prior to milking (97%) and the majority of farmers dry the teats as well (71.2%). Only 13.5% follow post dip procedures and 24.5% said they performed some type of mastitis check. The large majority of farmers used plastic containers to store and transport milk to the collection centers (92%). Approximately three fourths of farmers used hot water to clean the collection containers and 90% used detergent. The most common source of water was tap (77%) but 23% of farmers used well or river water. While 71.7% of farmers stored milk in closed containers only
14% stored milk in cold water prior to transportation to the collection center. Milk was most often taken to the collection center twice a day, but 34.2% of farmers only took their milk once a day. Of this 34.2%, some used the evening milk for home consumption while some stored the milk overnight and combined the evening milk with the morning milk to be taken to the collection center. Generally there was less than hour between the when cows were milked and when the milk arrived at the collection center, but a little over 20% of farmers responded that the time was over 12 hours. On average, the travel time to the collection center was 15.2 minutes. Most farmers (65.5%) consume a small portion of milk at home and some (18.6%) sell a portion to neighbors meaning most milk is brought to the collection centers. On average, farmers bring 15.6L to the collection centers per day (Table 7).

Using the Fisher’s exact test, the following differences between peri-urban and urban farms were statistically significant with p<.10; urban farms were more likely to have one individual assigned only to milking (p=0.0531) and to sell their milk to other farmers (p=.0302), and peri-urban farms were more likely to use hot water to clean the collection containers (p=0.0400). There was also a significant difference in where cows were kept between peri-urban and urban farms (p=0.0011). All urban farms kept cows in a tie stall and in peri-urban farms the cows were kept either in a tie stall, free stall, pasture, or a combination of the three. Based on the Wilcoxon rank sum test, there was a statistically significant difference in the number of exotic cows between peri-urban and urban farms (p=.0314). On average, urban farms owned more exotic cows. There was no significant difference between peri-urban and urban farms for all other variables.

Using simple logistic regression the following factors were found to be associated with S. aureus recovery from milk samples: new cows having a pre-test of any type prior to entering the herd (p=0.0925), administration of vaccines at least once a year (p=0.066), assigning one
individual to only milk (0.0924), and the total number of liters farmers bring to the collection center per day (0.043). The odds of recovering *S. aureus* from milk samples where farmers administered a pre test were 2.27 times that of farmers who did not. Similarly, the odds of *S. aureus* recovery from milk originating on farms where vaccines were administered to cows at least once a year were five times that of milk from farms where no vaccines were administered. The odds of *S. aureus* recovery for samples whose milker performs other duties in addition to milking were 3.21 times that of samples whose milkers performed other duties as well. For every one additional liter of milk a farmer brought to the collection center the odds of *S. aureus* recovery increased by 4.3%. There was no significant association between the different regions collected from and the recovery of *S. aureus* from milk (Table 8). The following variables were omitted from the statistical analysis due to too few observations resulting in unstable results; containers, pre-dip, and post-dip.

After using backwards selection, administration of vaccines at least once a year was no longer predictive of *S. aureus* recovery at p<0.10. The final model consisted of the amount of liters brought to the collection center per day, whether or not pre-tests were performed on new cows prior to entering the herd and the assignment of an individual to only milk. Once the predictor variables were placed in the same model, the associations slightly changed. The odds ratio for liters of milk brought to the collection center per day increased to 1.05 and the odds ratio for the assignment of an individual to only milk increased to 4.71. The odds ratio for administering a pre-test changed to 0.31 showing more of an association (Table 9).
Discussion

Most studies concerning *S. aureus* in bovine milk in Ethiopia are related to the prevalence of mastitis and do not focus on the public health aspect of the pathogen. Our study looked at milk after transportation to the collection center to account for both *S. aureus* strains introduced through the cow as well as through human and environmental contamination. The overall prevalence of *S. aureus* was 26% for individual farmer’s bulk milk and over 50% for combined bulk tanks for both times sampled. While the prevalence of *S. aureus* varies greatly throughout different countries and production systems, the results of this study appear to be lower than many others. A study in Brazil found *S. aureus* in 70.4% of the unpasteurized bovine milk samples$^{125}$ and a study looking at raw bovine milk in Zimbabwe found a prevalence of 58.3%$^{126}$. Although lower than other countries, a 26% prevalence of *S. aureus* poses a risk to consumers based on the unavailability of refrigeration capabilities in Ethiopia and the heat stability of staphylococcal enterotoxins.

The prevalence did vary slightly between the different geographical regions, but the results from ordinary logistic regression showed there was not a statistically significant difference. This is consistent with the mostly homogenous nature of the questionnaire results indicating similar milking and hygiene practices among all regions. Even so, the sample size for urban regions was small in comparison to peri-urban regions. In the future, more collection centers should be sampled from urban regions to determine the differences more accurately.

*Salmonella* was not found in any of the milk samples. Based on our results, it is possible that milk on peri-urban and urban farms in central Ethiopia was not contaminated with *Salmonella*. Another possibility is that the methods used were not sensitive enough to detect
the presence of the pathogen or that the sample size was not large enough. Previous studies have shown a low prevalence of *Salmonella* in cattle in Ethiopia. A study conducted on 450 cattle from Addis Ababa, Ethiopia, and surrounding areas isolated *Salmonella* from only 8 cows (1.8%)\(^{96}\). Even with the low prevalence found within cattle in Ethiopia, isolates of *Salmonella* have been recovered from milk and milk products\(^{97}\). As the dairy industry continues to grow and moves towards a more intensive production system, it is important to maintain prevalence data for *Salmonella* in raw milk.

Among *S. aureus* isolates from individual farmers, 87% were resistance to at least one antimicrobial and 25% were multiply resistant. Approximately 80% of *S. aureus* isolates from combined bulk tanks were resistant to one or more antimicrobials and 47% were multiply resistant. *S. aureus* isolates were most commonly resistant to penicillin (74.2%), tetracycline (32.3%), and ampicillin (3.2%). Other studies show similar results among *S. aureus* strains isolated from bovine mastitic milk. Studies throughout Ethiopia isolating *S. aureus* from bovine mastitic milk show high levels of resistance to ampicillin, amoxicillin, penicillin, polymixin B, oxytetracycline, sulfamethoxazole, and streptomycin\(^{89,90}\). A larger sample size is needed to determine if there are differences between antimicrobial resistance patterns among different regions. Although the questionnaire addressed the use of antimicrobials, nearly all farmers were unaware of the specific types and frequency. This information combined with a larger sample size would help determine relationships between antimicrobial use and resistance patterns.

Looking just at *S. aureus* strains isolated from individual farmers, 29% were found to have one or more SE gene with sea and sec-1 being the most frequent. The frequency with which enterotoxigenic *S. aureus* was found in bovine milk in past studies is highly variable. Looking at studies identifying only the classic SE genes, the range was from 13.3% to
S. aureus from bovine unpasteurized milk in Brazil were consistent with sea and sec being the most prevalent. Other studies found genes sea and sed most frequently in S. aureus strains recovered from bovine milk and SEA, SEB, and SEC in bovine mastitic milk. The tst gene was not found in any isolates, however the positive control failed and it cannot be concluded that the tst gene was absent in the S. aureus strains isolated. A study in Tennessee looking for toxic shock syndrome toxin genes in bovine mastitic milk found the tsst-1 gene in 25.6% of the S. aureus stains isolated. Additional studies should look for the presence of seg and sei genes based on the ability of the associated SEs to elicit emetic response in the primate model. It would also be beneficial to accomplish additional laboratory procedures to quantify the amount and types of SEs actually present in the milk.

PFGE analysis determined four separate clonal types that were clustered geographically and also found several sporadic isolates. While this is the first study of its kind in Ethiopia, the results are consistent with similar studies done in other areas concerning mastitis caused by S. aureus. In general, a limited number of bovine S. aureus clones with a broad geographic distribution are responsible for a large number of mastitis cases. Based on the results, there may be cross contamination between farms and/or the collection center may be a common source of contamination. Additionally, the Selale and Asela clusters contained multiple collection centers which are separated by several kilometers. Based on this, it is possible that collection containers provided by the co-operative to collect the combined bulk tank milk for transportation are a source of cross contamination. Further studies should look at this process to see if the collection containers are truly a source of cross contamination. If this is the case, a system should be developed to ensure collection containers are cleaned properly and handled in a way to prevent the spread of pathogens from collection center to collection center.
The questionnaire results mainly gave a broad understanding of the milking and hygiene practices among peri-urban and urban farms throughout central Ethiopia, but there were some differences detected between regions as well as risk factors identified. Urban farms were more associated with having one person assigned to only milk, selling milk to other farmers, and having exotic cows. The basis for the difference in milking assignment is not clear, but there may be a difference in the training provided in the regions or in social structures. In Akaki, Debre Zeit, and Asela, nearly half of the farmers sold their milk to other farmers, whereas in Selale, only 6.6% sold milk to other farmers. This may reflect the overall strength of the co-operative union in the region or the prices received by the farmers through the informal market. The higher number of exotic cows in urban regions compared with peri-urban regions is consistent with other surveys taken. Approximately 47% of all improved breeds of cattle are in Addis Ababa. A greater proportion of peri-urban farms used hot water to clean collection containers than urban farms. This may be due to a difference in hygiene awareness or access to equipment and supplies needed to produce hot water. Urban farms only kept their cows in a tie stall, however, peri-urban farms either kept cows in a tie stall, free stall, on pasture, or a combination of the three. This may be due to limited amount of space urban farmers have compared to peri-urban farmers.

The final model used in estimating the odds of recovery of *S. aureus* from milk in peri-urban and urban farms throughout central Ethiopia consisted of assigning an individual to only milk, amount of liters produced per day, and administration of a pre-test. Farms where one person was not assigned to only milk were more associated with *S. aureus* recovery. Assigning an individual to only milk is an indicator of good hygiene and may be indicative of other hygiene activities that help prevent the contamination of milk with *S. aureus*. In past studies, milking procedures such as udder preparation, pre-milking and post-milking teat disinfection, and
management of cows with mastitis were found to be associated with low somatic cell counts in bulk milk. Increased production and a larger herd size are often associated with lower somatic cell count numbers in developed countries. Farmers with larger herd size in the U.S. are thought to have better mastitis control\textsuperscript{131,132}. In the current study, increased production was associated with recovery of \textit{S. aureus}. The increased production in these cases was most likely due to more cows being milked and a larger herd size rather than individual cows being more productive. The results from the questionnaire showed that overall there is limited mastitis control. The association between administration of a pre-test and \textit{S. aureus} recovery was not as expected. Farmers who administered a pre-test were more associated with \textit{S. aureus} recovery than farmers did not. Generally this is a characteristic of farmers who are more mindful of the health of their cattle; however, the association may be measuring another factor. For example, farmers that performed a pre-test may have had more issues with the health of their herd or the quality of their milk in the past due to poor hygiene but attributed it to new cows. Also, it was not clear what a pre-test consisted of. Many farmers answered that their cows were given a general examination but did not know what exactly was checked for.
Limitations

The results from this study are representative of peri-urban and urban small-scale dairy farmers throughout central Ethiopia who participate in the formal market through dairy cooperatives and milk collection centers. Based on the nature of the study, there were a few limitations with the administration of the questionnaire and sampling that are unlikely to alter the overall results but should be mentioned.

Due to the multiple languages in the different regions sampled, additional translation was needed from either a co-operative manager or a quality control officer from the co-operative. At times, the milk collection center owner was present while farmers were questioned and in every case other farmers were observing the administration of the questionnaire. Additionally, convenient samples were taken from volunteers. All of these are potential sources of bias and may have influenced the way the farmers answered. Because the results regarding milking practices and hygiene measures were consistent with other surveys administered in the area, we believe the impact of the bias is negligible.
Recommendations

There are many factors that need to be addressed in order to enable urban and peri-urban dairy farmers to produce quality milk. The results from this study identify some areas throughout the milking process that should be addressed and can feasibly be changed. It is clear that the farmers in the study were knowledgeable on some hygienic practices, such as sanitizing the teats prior to milking and cleaning the collection container with detergent and hot water. However, almost 30% of the farmers did not dry the teats prior to milking and over 80% did not sanitize the teats after milking. Furthermore, the response to this question on the survey was often, “What is the point of cleaning the teats after milking when the cow is just going to lie down again and get dirty?” Additionally, over 80% of the farms surveyed do not have an individual assigned to only milk which was shown to be predictive of S. aureus recovery.

Regarding mastitis control, there is very little knowledge on what mastitis is and how to check for it. Of those who were aware of what mastitis is, over three-fourths did not check for it.

In order to help remedy the knowledge gaps it would be beneficial for dairy co-operatives to develop a protocol for hygienic milking that is specific to the farms within their region and also hold training sessions to make sure the farmers are aware of why each step is important. It is essential that the farmers have the proper inputs to implement the protocol set forth as well as an incentive to invest in these inputs and follow the protocol. While a quality based payment system is the ideal incentive for farmers to produce quality milk, it requires the cooperation and coordination from all levels of the milk supply chain and thus will be a lengthy process. In the mean time, incentives can be given at the co-operative level and even at the collection centers. While many of the collection centers surveyed gave advice to farmers on quality control, only
two provided small awards for quality milk. An incentive program should be developed at the co-operative level. Once an incentive program is developed and implemented it should be evaluated to see if it does foster higher quality milk among those collection centers who participate.
References


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57. FAO. Countries; Ethiopia, 2011.


60. Boutayeb A. The double burden of communicable and non-communicable diseases in developing countries. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2006;100:191-199.


113. CDC. MRSA Infections: Centers for Disease Control and Prevention, 2010.


Appendix A: Questionnaire

Date of collection __________

Cooperative site:  N. Sellale: □  S. Sellale: □  Assela: □

Individual Farm:  AA-1  AA-2  AA-3  AA-4  AA-5  DZ-1  DZ-2  DZ-3  DZ-4  DZ-5

Collection Ctr. Name/ no. ______ Bulk Tank? Yes: □  No: □
If no, Farm Name ______________________________________________

Contact person (address and Tel.): __________________________________________

Do you want to be informed of the results from this investigation?  Yes: □  No: □

I) Farm related questions

1. Estimated distance of farm from collection center: ______________

2. How long did it take to reach the center: __________________________

   a. Type of container: Plastic: □  Stainless Steel: □  Other: □

   b. How often do you bring milk to the collection center?

      Twice a day: □  Once a Day: □  Once every other day: □  Other: □

   c. How do you store the milk until it is transported to the collection center?

      Refrigerate: □  Open: □  Closed: □  Other: □

   d. How long did it take for the milk to get to the collection center since the cow(s)
      was(ware) milked?

      Less than 6 hours: □  Between 6 and 12 hours: □  Greater than 12 hours □

3. How many dairy cows are in the herd? ______________

   a. How many of each breed?  Local: □  Exotic: □  Mixed: □

   b. From how many cows on the farm was the milk pooled from? □
4. How many liters of milk are brought into the collection center each time? ________
   a. What percent is kept at farm for consumption? ________%  
   b. What percent does farmer sell on own for consumption? ________%

5. What is the method of milking? Hand: [ ]  Machine: [ ]  Other: [ ]

6. Hygiene:
   a. Source of water:  Tap: [ ]  Well: [ ]  Other: [ ]
   b. How is collection container cleaned:
      Disinfectants: [ ]  Water only: [ ]  Access to hot water: [ ]

7. What hygiene procedures are used before and after milking?
   a. Pre dip: [ ]  If yes what is used? ________
   b. Post dip: [ ]  If yes what is used? ________
   c. Is there a check for mastitis?  Yes: [ ]  No: [ ]
      i. Did any of your cows have mastitis?
         For this milking: Yes: [ ]  No: [ ]  If yes, how many: [ ]
         In the last 6 months: Yes: [ ]  No: [ ]  If yes, how many: [ ]
      ii. Is that milk used? Yes: [ ]  No: [ ]  If yes, purpose: ________
      iii. How do you treat for mastitis? ________________________________
   d. Are any antibiotics used to treat cows? ___________________________
   e. Where are cows kept? Pasture: [ ]  Free stalls: [ ]  Tie Stalls: [ ]  Other: [ ]

8. Is there any tests/surveillance for diseases before new cows interact with the rest of the herd?
   Yes: [ ]  No: [ ]  If yes, what: ________________________________
9. How often do cows get checked for diseases?
   - Once a month: 
   - Every quarter: 
   - Random: 

10. What routine vaccinations are the cows exposed to?
    - Type: 
    - How often: 

11. Are any records kept?
    - Yes: 
    - No: 
    - If yes, for what? 
    - How often: 

12. How many people work on the dairy?
    - Milkers: 
    - Manure management: 
    - Mixed: 
    - Other: 

II) Collection center/ bulk tank related questions

13. Is there a system in place to monitor the temperature of the bulk tanks?
    - Yes: 
    - No: 
    - If yes, are records kept? 

14. Are there quality checks?
    - Lactometer: 
    - Alcohol Test: 
    - If yes for any, how often? 

15. Are there standards at which the milk is not used? 

16. How often does the bulk tank get cleaned? 

17. What is the source of water:
    - Tap: 
    - Well: 
    - Other: 

18. What is the method of disinfection? 

19. How long does the milk stay at collection center? 

20. What happens to the collected milk? (if mixed activities, use % distribution)
    a. Pasteurization % Where? 
    b. Cheese preparation % Where? 
    c. On-site sale for consumption? % 
    d. Other? Where? 

75
21. Is there pay incentive to the farmers for providing good quality milk? Or penalty for rancid or low quality?

22. On average, how many farmers bring milk to your collection center per or perhaps better per collection cycle:

   Morning: [ ]  
   Evening: [ ]
Appendix B: Tables

Table 1: Sequences, adopted from Johnson\textsuperscript{123}, Oliveira and Lancastre\textsuperscript{121}, and Brakstad\textsuperscript{122} used in multiplex PCR for identifying the mecA, nuc, and staphylococcal enterotoxin genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5'-3')</th>
<th>Amplicon size (bp)</th>
<th>Location within Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>SEA - F</td>
<td>ttggaaacggttaaacgaa</td>
<td>120</td>
<td>490-509</td>
</tr>
<tr>
<td></td>
<td>SEA - R</td>
<td>gaaaccttcccatcaaaaca</td>
<td></td>
<td>591-610</td>
</tr>
<tr>
<td>seb</td>
<td>SEB - F</td>
<td>tgcactaaactgcaaaacg</td>
<td>478</td>
<td>634-653</td>
</tr>
<tr>
<td></td>
<td>SEB - R</td>
<td>gcaggtactctataagtgcc</td>
<td></td>
<td>1091-1110</td>
</tr>
<tr>
<td>sec-1</td>
<td>SEC - F</td>
<td>gacataaaagctagaaat</td>
<td>257</td>
<td>676-695</td>
</tr>
<tr>
<td></td>
<td>SEC - R</td>
<td>aatcggattaaacatttcc</td>
<td></td>
<td>913-932</td>
</tr>
<tr>
<td>sed</td>
<td>SED - F</td>
<td>ctgctttagaatctcttcct</td>
<td>317</td>
<td>354-373</td>
</tr>
<tr>
<td></td>
<td>SED - R</td>
<td>taatgcctatatcctataagg</td>
<td></td>
<td>652-671</td>
</tr>
<tr>
<td>see</td>
<td>SEE - F</td>
<td>taatgaatgacgaaaaacg</td>
<td>170</td>
<td>491-510</td>
</tr>
<tr>
<td></td>
<td>SEE - R</td>
<td>taactttacgtggaccccttc</td>
<td></td>
<td>640-659</td>
</tr>
<tr>
<td>tst-1</td>
<td>TST - F</td>
<td>atgggcactacgcttgata</td>
<td>350</td>
<td>251-270</td>
</tr>
<tr>
<td></td>
<td>TST - R</td>
<td>ttcttctacaaccctgttt</td>
<td></td>
<td>581-600</td>
</tr>
<tr>
<td>nuc</td>
<td>NUC - F</td>
<td>gcgatgtgatgtgatacggti</td>
<td>270</td>
<td>48-70</td>
</tr>
<tr>
<td></td>
<td>NUC - R</td>
<td>agccacaggcctgactaaacgc</td>
<td></td>
<td>303-328</td>
</tr>
<tr>
<td>meca</td>
<td>MECA - F</td>
<td>tccagattacaaccttccccagg</td>
<td>162</td>
<td>1190-1211</td>
</tr>
<tr>
<td></td>
<td>MECA - R</td>
<td>caacctcatatcctgttaacg</td>
<td></td>
<td>1351-1332</td>
</tr>
</tbody>
</table>

* Primers synthesized by Integrated DNA Technologies (IDT, Coralville, IA)

Table 2: Prevalence of S. aureus in individual bulk tanks

<table>
<thead>
<tr>
<th>Geographic Location</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selale (n=85)</td>
<td>24 (28.2%)</td>
</tr>
<tr>
<td>Asela (n=21)</td>
<td>4 (19.0%)</td>
</tr>
<tr>
<td>Debre Zeit (n=5)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Akaki (n=8)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Urban (n=13)</td>
<td>3 (23.1%)</td>
</tr>
<tr>
<td>Peri-Urban (n=106)</td>
<td>28 (26.4%)</td>
</tr>
<tr>
<td>Total (n=119)</td>
<td>31 (26.1%)</td>
</tr>
</tbody>
</table>

* Urban Includes both Akaki and Debre Zeit

1 Peri-Urban Includes both Selale and Asela

*Procedures to isolate Salmonella enterica were also performed, however all samples were negative.
### Table 3: Prevalence of S. aureus in combined bulk tanks

<table>
<thead>
<tr>
<th>Geographic Location</th>
<th>Sampling Rotation 1</th>
<th>Sampling Rotation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selale (n=10)</td>
<td>6 (60%)</td>
<td>Selale (n=10)</td>
</tr>
<tr>
<td>Asela (n=3)</td>
<td>1 (33.3%)</td>
<td>Asela (n=3)</td>
</tr>
<tr>
<td>Debre Zeit (n=1)</td>
<td>0 (0.0%)</td>
<td>Debre Zeit</td>
</tr>
<tr>
<td>Akaki (n=2)</td>
<td>1 (50%)</td>
<td>Akaki</td>
</tr>
</tbody>
</table>

*Urban (n=3)        | 1 (33.3%)           | *Urban               | Not done            |
*Peri-Urban (n=13)  | 7 (53.8%)           | *Peri-Urban (n=13)  | 7 (53.8%)           |
Total (n=16)        | 8 (50%)             | Total (n=13)         | 7 (53.8%)           |

*Urban includes both Akaki and Debre Zeit
*Peri-Urban includes both Selale and Asela
*Procedures to isolate *Salmonella enterica* were also performed, however all samples were negative.

### Table 4: Prevalence of antimicrobial resistance among S. aureus isolates from individual bulk tanks

<table>
<thead>
<tr>
<th>Geographic Location</th>
<th>Ampicillin</th>
<th>Penicillin</th>
<th>Tetracycline</th>
<th>Pan-susceptible</th>
<th>Resistant to ≥ 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selale (n=24)</td>
<td>1 (4.2%)</td>
<td>21 (87.5%)</td>
<td>5 (20.8%)</td>
<td>3 (12.5%)</td>
<td>21 (87.5%)</td>
</tr>
<tr>
<td>Asela (n=4)</td>
<td>0 (0.0%)</td>
<td>1 (25%)</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>Debre Zeit (n=2)</td>
<td>0 (0.0%)</td>
<td>1 (50%)</td>
<td>2 (100%)</td>
<td>0 (0.0%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Akaki (n=1)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (100%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Urban (n=3)</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
<td>3 (66.6%)</td>
<td>1 (33.3%)</td>
<td>2 (66.6%)</td>
</tr>
<tr>
<td>Peri-Urban (n=28)</td>
<td>1 (3.6%)</td>
<td>22 (78.6%)</td>
<td>7 (25%)</td>
<td>4 (14.3%)</td>
<td>25 (89.3%)</td>
</tr>
<tr>
<td>Total (n=31)</td>
<td>1 (3.2%)</td>
<td>23 (74.2%)</td>
<td>10 (32.3%)</td>
<td>5 (16.1%)</td>
<td>27 (87.1%)</td>
</tr>
</tbody>
</table>

*All isolates from individual farmers’ bulk tanks were susceptible to all other antibiotics tested: Erythromycin, Streptomycin, Oxacillin, Amox/Clav, Trimethoprim/sulfamethoxazole, Ceftriaxone, Vancomycin, and Gentamicin.

### Table 5: Resistance patterns of S. aureus isolates

<table>
<thead>
<tr>
<th>Location of Isolates</th>
<th>Pn</th>
<th>Te</th>
<th>TePn</th>
<th>AmPn</th>
<th>Pan-susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selale (n=24)</td>
<td>15 (62.5%)</td>
<td>0 (0%)</td>
<td>5 (20.8%)</td>
<td>1 (4.2%)</td>
<td>3 (12.5%)</td>
</tr>
<tr>
<td>Asela (n=4)</td>
<td>2 (50%)</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Debre Zeit (n=2)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Akaki (n=1)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Urban (n=3)</td>
<td>0 (0%)</td>
<td>1 (33.3%)</td>
<td>1 (33.3%)</td>
<td>0 (0%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Peri-Urban (n=28)</td>
<td>17 (60.7%)</td>
<td>0 (0%)</td>
<td>6 (21.4%)</td>
<td>1 (3.6%)</td>
<td>4 (14.3%)</td>
</tr>
<tr>
<td>Total (n=31)</td>
<td>17 (54.8%)</td>
<td>1 (3.2%)</td>
<td>7 (22.6%)</td>
<td>1 (3.2%)</td>
<td>5 (16.1%)</td>
</tr>
</tbody>
</table>

*Urban includes both Akaki and Debre Zeit
*Peri-Urban includes both Selale and Asela
Table 6: Prevalence of Staphylococcal Enterotoxins (SE) genes from individual farmer’s bulk tank isolates

<table>
<thead>
<tr>
<th>Location of Isolates</th>
<th>sea</th>
<th>seb</th>
<th>sec-1</th>
<th>sed</th>
<th>≥ 1 se*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selale (n=24)</td>
<td>5 (20.8%)</td>
<td>1 (4.2%)</td>
<td>2 (8.3%)</td>
<td>1 (4.2%)</td>
<td>6 (25%)</td>
</tr>
<tr>
<td>Asela (n=4)</td>
<td>1 (25%)</td>
<td>0 (0.0%)</td>
<td>1 (25%)</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>Debre Zeit (n=2)</td>
<td>1 (50%)</td>
<td>0 (0.0%)</td>
<td>1 (50%)</td>
<td>0 (0.0%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>Akaki (n=1)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Urban (n=3)</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Peri-Urban (n=28)</td>
<td>6 (21.4%)</td>
<td>1 (3.6%)</td>
<td>3 (10.7%)</td>
<td>3 (10.7%)</td>
<td>8 (28.6%)</td>
</tr>
<tr>
<td>Total (n=31)</td>
<td>7 (22.6%)</td>
<td>1 (3.2%)</td>
<td>4 (12.9%)</td>
<td>3 (9.7%)</td>
<td>9 (29.0%)</td>
</tr>
</tbody>
</table>

*PCR was also performed for the presence of tst with no positive results however our positive control did not work so the non-presence of tst cannot be confirmed.
*PCR was also performed for the see gene but all isolates were negative.

Table 7: Stratified Questionnaire Results (refer to Appendix C for full questions)

<table>
<thead>
<tr>
<th>Question</th>
<th>Selale</th>
<th>Asela</th>
<th>Debre Zeit</th>
<th>Akaki</th>
<th>Urban</th>
<th>Peri-Urban</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liters of milk per CC (sd)</td>
<td>(n=10)</td>
<td>(n=3)</td>
<td>(n=1)</td>
<td>(n=2)</td>
<td>(n=4)</td>
<td>(n=13)</td>
<td>(n=16)</td>
</tr>
<tr>
<td></td>
<td>869 (466.7)</td>
<td>111.7 (95.4)</td>
<td>400 (---)</td>
<td>750 (636.4)</td>
<td>633.3 (493.3)</td>
<td>694.2 (524.6)</td>
<td></td>
</tr>
<tr>
<td>Number of farmers per CC (sd)</td>
<td>(n=10)</td>
<td>(n=3)</td>
<td>(n=1)</td>
<td>(n=2)</td>
<td>(n=4)</td>
<td>(n=13)</td>
<td>(n=16)</td>
</tr>
<tr>
<td></td>
<td>101.8 (28.7)</td>
<td>24.3 (9.5)</td>
<td>40 (---)</td>
<td>91 (83.4)</td>
<td>55.5 (65.9)</td>
<td>83.9 (42.3)</td>
<td></td>
</tr>
<tr>
<td>Time milk kept at CC (sd)</td>
<td>(n=10)</td>
<td>(n=3)</td>
<td>(n=1)</td>
<td>(n=2)</td>
<td>(n=3)</td>
<td>(n=11)</td>
<td>(n=14)</td>
</tr>
<tr>
<td></td>
<td>44.25 (19.5)</td>
<td>30 (---)</td>
<td>60 (21.2)</td>
<td>45 (21.2)</td>
<td>50 (17.3)</td>
<td>43 (19.0)</td>
<td></td>
</tr>
<tr>
<td>Travel Time (sd)</td>
<td>(n=76)</td>
<td>(n=21)</td>
<td>(n=4)</td>
<td>(n=8)</td>
<td>(n=12)</td>
<td>(n=97)</td>
<td>(n=111)</td>
</tr>
<tr>
<td></td>
<td>16.4 (19.5)</td>
<td>11.4 (5.5)</td>
<td>6.1 (4.5)</td>
<td>19.9 (11.7)</td>
<td>15.3 (11.8)</td>
<td>15.3 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Cows per herd (sd)</td>
<td>(n=75)</td>
<td>(n=24)</td>
<td>(n=4)</td>
<td>(n=8)</td>
<td>(n=12)</td>
<td>(n=99)</td>
<td>(n=113)</td>
</tr>
<tr>
<td></td>
<td>3.28 (1.8)</td>
<td>2.9 (1.36)</td>
<td>2 (1.4)</td>
<td>4 (3.2)</td>
<td>3.3 (2.8)</td>
<td>3.2 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Cows milked (sd)</td>
<td>(n=65)</td>
<td>(n=24)</td>
<td>(n=4)</td>
<td>(n=8)</td>
<td>(n=12)</td>
<td>(n=89)</td>
<td>(n=102)</td>
</tr>
<tr>
<td></td>
<td>2.35 (1.3)</td>
<td>1.3 (0.5)</td>
<td>2 (1.4)</td>
<td>2.25 (1.9)</td>
<td>2.2 (1.7)</td>
<td>2.1 (1.25)</td>
<td></td>
</tr>
<tr>
<td>Mixed cows (sd)</td>
<td>(n=76)</td>
<td>(n=22)</td>
<td>(n=4)</td>
<td>(n=8)</td>
<td>(n=12)</td>
<td>(n=98)</td>
<td>(n=112)</td>
</tr>
<tr>
<td></td>
<td>1.9 (.7)</td>
<td>1.6 (.13)</td>
<td>0 (---)</td>
<td>2.4 (3.3)</td>
<td>1.6 (2.9)</td>
<td>1.8 (1.62)</td>
<td></td>
</tr>
<tr>
<td>Local cows (sd)</td>
<td>(n=76)</td>
<td>(n=22)</td>
<td>(n=4)</td>
<td>(n=8)</td>
<td>(n=12)</td>
<td>(n=98)</td>
<td>(n=112)</td>
</tr>
<tr>
<td></td>
<td>.7 (.75)</td>
<td>.6 (.143)</td>
<td>0 (---)</td>
<td>0 (---)</td>
<td>.7 (.17)</td>
<td>.6 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Exotic cows (sd)</td>
<td>(n=76)</td>
<td>(n=22)</td>
<td>(n=4)</td>
<td>(n=8)</td>
<td>(n=12)</td>
<td>(n=98)</td>
<td>(n=112)</td>
</tr>
<tr>
<td></td>
<td>.7 (.13)</td>
<td>.6 (.13)</td>
<td>2 (1.4)</td>
<td>1.5 (2.8)</td>
<td>1.7 (2.4)</td>
<td>.8 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Total liters per farmer (sd)</td>
<td>(n=75)</td>
<td>(n=24)</td>
<td>(n=4)</td>
<td>(n=8)</td>
<td>(n=12)</td>
<td>(n=99)</td>
<td>(n=113)</td>
</tr>
<tr>
<td></td>
<td>34.5 (9.7)</td>
<td>8.4L (8.23)</td>
<td>17.8L (3.3)</td>
<td>18.2L (16.4)</td>
<td>18L (13.2)</td>
<td>15.3L (10.1)</td>
<td></td>
</tr>
<tr>
<td>Workers per farm (sd)</td>
<td>(n=73)</td>
<td>(n=24)</td>
<td>(n=4)</td>
<td>(n=8)</td>
<td>(n=12)</td>
<td>(n=97)</td>
<td>(n=109)</td>
</tr>
<tr>
<td></td>
<td>2.8 (1.6)</td>
<td>2.2 (1.4)</td>
<td>1.75 (1.0)</td>
<td>2 (0.8)</td>
<td>1.9 (0.8)</td>
<td>2.7 (1.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Continue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of cows</td>
<td>P</td>
<td>(n=74)</td>
<td>24.3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>(n=24)</td>
<td>23%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>TS</td>
<td>(n=4)</td>
<td>28.4%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>(n=8)</td>
<td>24.3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Milk sold</td>
<td>No</td>
<td>(n=76)</td>
<td>93.4%</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>6.6%</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Milk kept for</td>
<td>No</td>
<td>(n=76)</td>
<td>40.8%</td>
<td>75%</td>
<td>12.5%</td>
<td>33.3%</td>
<td>66.7%</td>
</tr>
<tr>
<td>home cons.</td>
<td>Yes</td>
<td>(n=23)</td>
<td>59.2%</td>
<td>25%</td>
<td>87.5%</td>
<td>35.4%</td>
<td>64.6%</td>
</tr>
<tr>
<td>Container</td>
<td>No</td>
<td>(n=76)</td>
<td>10.5%</td>
<td>75%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
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<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>89.5%</td>
<td>25%</td>
<td>100%</td>
<td>83.3%</td>
<td>92%</td>
</tr>
<tr>
<td>Time b/t milking and CC</td>
<td>No</td>
<td>(n=76)</td>
<td>30.3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>69.7%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Milk transported in closed container</td>
<td>No</td>
<td>(n=75)</td>
<td>40%</td>
<td>0%</td>
<td>12.5%</td>
<td>8.3%</td>
<td>91.7%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>60%</td>
<td>100%</td>
<td>100%</td>
<td>87.5%</td>
<td>91.7%</td>
</tr>
<tr>
<td>Milk stored in cold water</td>
<td>No</td>
<td>(n=77)</td>
<td>81.8%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=25)</td>
<td>18.2%</td>
<td>0%</td>
<td>12.5%</td>
<td>8.3%</td>
<td>91.7%</td>
</tr>
<tr>
<td>Often milk brought to CC</td>
<td>1/day</td>
<td>No</td>
<td>(n=76)</td>
<td>39.5%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>65.8%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Source of water</td>
<td>Tap</td>
<td>(n=24)</td>
<td>65.8%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>(n=4)</td>
<td>11.8%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>River</td>
<td>(n=4)</td>
<td>22.4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Hot water for cleaning</td>
<td>No</td>
<td>(n=75)</td>
<td>16%</td>
<td>100%</td>
<td>20%</td>
<td>55.6%</td>
<td>44.4%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=23)</td>
<td>84%</td>
<td>0%</td>
<td>80%</td>
<td>55.6%</td>
<td>44.4%</td>
</tr>
<tr>
<td>Detergent to clean collection containers</td>
<td>No</td>
<td>(n=76)</td>
<td>5.3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>94.7%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Pre Dip</td>
<td>No</td>
<td>(n=78)</td>
<td>3.8%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>96.2%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Dry</td>
<td>No</td>
<td>(n=73)</td>
<td>31.5%</td>
<td>0%</td>
<td>25%</td>
<td>16.7%</td>
<td>63.3%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>68.5%</td>
<td>100%</td>
<td>75%</td>
<td>33.3%</td>
<td>30.9%</td>
</tr>
<tr>
<td>Post Dip</td>
<td>No</td>
<td>(n=75)</td>
<td>90.7%</td>
<td>100%</td>
<td>74.8%</td>
<td>80%</td>
<td>87.9%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>(n=24)</td>
<td>9.3%</td>
<td>0%</td>
<td>25.2%</td>
<td>20%</td>
<td>12.1%</td>
</tr>
<tr>
<td>Mastitis Check</td>
<td>No</td>
<td>(n=72)</td>
<td>75%</td>
<td>100%</td>
<td>50%</td>
<td>66.7%</td>
<td>76.1%</td>
</tr>
<tr>
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<td>Yes</td>
<td>(n=20)</td>
<td>25%</td>
<td>0%</td>
<td>50%</td>
<td>33.3%</td>
<td>23.9%</td>
</tr>
<tr>
<td>Antibiotic use</td>
<td>No</td>
<td>(n=66)</td>
<td>24.2%</td>
<td>75%</td>
<td>12.5%</td>
<td>33.3%</td>
<td>21.3%</td>
</tr>
<tr>
<td></td>
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<td>(n=23)</td>
<td>75.6%</td>
<td>25%</td>
<td>87.5%</td>
<td>66.7%</td>
<td>78.7%</td>
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### Table 7 Continued

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<td><strong>Routine check ups</strong></td>
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<td><strong>Separate Workers</strong></td>
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CC = Collection Center

### Table 8: Unadjusted associations between individual variables and the recovery of S. aureus

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<tr>
<th>Variable</th>
<th>Pos (mean)</th>
<th>Neg (mean)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liters of Milk per CC (n=16)</strong></td>
<td>(n=13) 732.7L (516.1)</td>
<td>(n=3) 466.7L (486.5)</td>
<td>1.00</td>
<td>(0.99, 1.00)</td>
<td>0.274</td>
</tr>
<tr>
<td>(sd)</td>
<td>(n=13) 85 (45.8)</td>
<td>(n=3) 69.3 (44.9)</td>
<td>1.01</td>
<td>(0.99, 1.04)</td>
<td>.364</td>
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<tr>
<td><strong>Farmers per CC (n=16)</strong></td>
<td>(n=11) 45.2min (16.8)</td>
<td>(n=3) 41.7min (21.6)</td>
<td>1.01</td>
<td>(0.95, 1.10)</td>
<td>0.776</td>
</tr>
<tr>
<td>(sd)</td>
<td>(n=25) 15.6min (19.4)</td>
<td>(n=84) 15.2min (16.3)</td>
<td>1.00</td>
<td>(0.97, 1.03)</td>
<td>0.909</td>
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<tr>
<td><strong>Time milk kept at CC (n=14)</strong></td>
<td>(n=25) 3.2 (1.7)</td>
<td>(n=86) 3.1 (1.8)</td>
<td>1.16</td>
<td>(0.91, 1.45)</td>
<td>0.223</td>
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<tr>
<td>(sd)</td>
<td>(n=23) 2.5 (1.0)</td>
<td>(n=78) 2 (1.4)</td>
<td>1.31</td>
<td>(0.93, 1.87)</td>
<td>0.117</td>
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<tr>
<td><strong>Cows per herd (n=113)</strong></td>
<td>(n=25) 0.6 (1.4)</td>
<td>(n=85) 0.6 (1.7)</td>
<td>1.02</td>
<td>(0.74, 1.31)</td>
<td>0.548</td>
</tr>
<tr>
<td>(sd)</td>
<td>(n=25) 0.9 (1.6)</td>
<td>(n=85) 0.7 (1.5)</td>
<td>1.11</td>
<td>(0.82, 1.46)</td>
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<tr>
<td><strong>Cows milked per herd (n=102)</strong></td>
<td>(n=25) 19.4L (12.5)</td>
<td>(n=86) 14.5L (9.6)</td>
<td>1.043</td>
<td>(1.00, 1.09)</td>
<td>0.043*</td>
</tr>
<tr>
<td>(sd)</td>
<td>(n=25) 0.6 (1.4)</td>
<td>(n=85) 0.6 (1.7)</td>
<td>1.02</td>
<td>(0.74, 1.31)</td>
<td>0.548</td>
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<tr>
<td><strong>Total liters per farmer (n=113)</strong></td>
<td>(n=25) 19.4L (12.5)</td>
<td>(n=86) 14.5L (9.6)</td>
<td>1.043</td>
<td>(1.00, 1.09)</td>
<td>0.043*</td>
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<tr>
<td>(sd)</td>
<td>(n=25) 0.6 (1.4)</td>
<td>(n=85) 0.6 (1.7)</td>
<td>1.02</td>
<td>(0.74, 1.31)</td>
<td>0.548</td>
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<tr>
<td>--------------------</td>
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<td></td>
</tr>
<tr>
<td><strong>Workers per farm (n=109)</strong></td>
<td>(n=23)</td>
<td>(n=86)</td>
<td>1.25</td>
<td>(0.93, 1.67)</td>
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<tr>
<td>(sd)</td>
<td>3.0 (1.6)</td>
<td>2.5 (1.4)</td>
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<tr>
<td><strong>Location of farms (n=123)</strong></td>
<td>Pos</td>
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<td>Odds Ratio</td>
<td>95% CI</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>94</td>
<td>1.797</td>
<td>(0.226, 11.52)</td>
<td>0.4459</td>
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<td>Debre Zeit (1)</td>
<td>2</td>
<td>3</td>
<td>0.385</td>
<td>(0.020, 2.34)</td>
<td>reference</td>
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<tr>
<td>Addis Ababa (2)</td>
<td>1</td>
<td>8</td>
<td>0.514</td>
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<td>Asela (3)</td>
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<td>Selale (4)</td>
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<tr>
<td><strong>Time b/t milking and CC (n=114)</strong></td>
<td>Pos</td>
<td>Neg</td>
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<td>p-value</td>
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<tr>
<td>&lt; 1 hour</td>
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<td>69</td>
<td>1.044</td>
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<td>0.4459</td>
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<tr>
<td>&gt;12 hours</td>
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<td>18</td>
<td>1.044</td>
<td>(0.226, 11.52)</td>
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<td><strong>Peri Urban/Urban (n=123)</strong></td>
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<td>Neg</td>
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<td>p-value</td>
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<td>Peri-Urban (1)</td>
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<td>94</td>
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<td>(0.305, 5.094)</td>
<td>0.9067</td>
</tr>
<tr>
<td>Urban (2)</td>
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<td>84</td>
<td>1.084</td>
<td>(0.305, 5.094)</td>
<td>0.9067</td>
</tr>
<tr>
<td><strong>Often (n=114)</strong></td>
<td>Pos</td>
<td>Neg</td>
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<td>95% CI</td>
<td>p-value</td>
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<td>Once a day (1)</td>
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<td>0.889</td>
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<td>Twice a day (2)</td>
<td>5</td>
<td>18</td>
<td>1.069</td>
<td>(0.409, 2.67)</td>
<td>0.889</td>
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<td>Odds Ratio</td>
<td>95% CI</td>
<td>p-value</td>
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<td>Open (0)</td>
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<td>(0.305, 5.094)</td>
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<td>24</td>
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<td>(0.305, 5.094)</td>
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<td>(0.125, 1.279)</td>
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<td>p-value</td>
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<td>Tap (1)</td>
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<td>(0.46, 15.58)</td>
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<td>River/stream (3)</td>
<td>4</td>
<td>13</td>
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<td>0.93, 1.67</td>
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<td><strong>Detergent (n=114)</strong></td>
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<td>Neg</td>
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<td>95% CI</td>
<td>p-value</td>
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<td>No (0)</td>
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<td>(0.022, 2.41)</td>
<td>0.366</td>
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<td><strong>Hot water (n=109)</strong></td>
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<td>p-value</td>
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<td>No (0)</td>
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<td>0.968</td>
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<td><strong>Dry (n=111)</strong></td>
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<td>Neg</td>
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<td>p-value</td>
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<td>(0.32, 2.40)</td>
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<td><strong>Mastitis Check (n=106)</strong></td>
<td>Pos</td>
<td>Neg</td>
<td>Odds Ratio</td>
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<td>p-value</td>
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<td>No (0)</td>
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<td>(0.49, 4.19)</td>
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<td>11</td>
<td>1.26</td>
<td>(0.49, 4.19)</td>
<td>0.679</td>
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<td><strong>Antibiotic Use (n=103)</strong></td>
<td>Pos</td>
<td>Neg</td>
<td>Odds Ratio</td>
<td>95% CI</td>
<td>p-value</td>
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<tr>
<td>No (0)</td>
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<td>0.571</td>
<td>(0.15, 1.74)</td>
<td>0.338</td>
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<tr>
<td>Yes (1)</td>
<td>4</td>
<td>19</td>
<td>0.571</td>
<td>(0.15, 1.74)</td>
<td>0.338</td>
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<td><strong>Location of cows (n=112)</strong></td>
<td>Pos</td>
<td>Neg</td>
<td>Odds Ratio</td>
<td>95% CI</td>
<td>p-value</td>
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<td>Pasture (1)</td>
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<td>86</td>
<td>0.929</td>
<td>(0.24, 3.59)</td>
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<td>Free Stall (2)</td>
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<td>20</td>
<td>0.929</td>
<td>(0.24, 3.59)</td>
<td>0.1596</td>
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Table 9: Logistic regression model for estimating the odds of *S. aureus* recovery among individual farmer’s bulk milk samples

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<th>Variable (n=102)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
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<td><strong>Liters per farmer</strong></td>
<td>1.05</td>
<td>(1.01 – 1.20)</td>
<td>0.03</td>
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<tr>
<td><strong>Pre-test</strong></td>
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<tr>
<td>No</td>
<td>0.31</td>
<td>(0.10 – 0.87)</td>
<td>0.026</td>
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<td>Yes</td>
<td>1.0</td>
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<tr>
<td><strong>Separate workers</strong></td>
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<tr>
<td>No</td>
<td>4.7</td>
<td>(1.10 – 33.9)</td>
<td>0.036</td>
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<td>Yes</td>
<td>1.0</td>
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Based on backward and forward selection, the model consists of liters per farmer, pre-test, and separate workers using p-values < .10.
### Appendix C: Explanation of Terms

<table>
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<tr>
<th>Term</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Peri Urban/Urban</td>
<td>Type of farm the farmer owns; peri-urban or urban</td>
</tr>
<tr>
<td>Location of Farms</td>
<td>Region the farmer is from; Selale, Asela, Debre Zeit, Akaki</td>
</tr>
<tr>
<td>Liters of milk per CC</td>
<td>Liters of milk collected per day</td>
</tr>
<tr>
<td>Farmers per CC</td>
<td>Number of farmers that bring milk to the collection center per day</td>
</tr>
<tr>
<td>Travel Time</td>
<td>Estimated time it took farmer to reach collection center in minutes</td>
</tr>
<tr>
<td>Time kept at CC</td>
<td>Time milk is kept at the collection center before transported elsewhere</td>
</tr>
<tr>
<td>Cows per herd</td>
<td>Number of dairy cows in the farmers herd</td>
</tr>
<tr>
<td>Cows milked</td>
<td>Number of dairy cows in herd that are actually milked</td>
</tr>
<tr>
<td>Mixed cows</td>
<td>Number of dairy cows in herd that are a cross between local and exotic breeds</td>
</tr>
<tr>
<td>Local cows</td>
<td>Number of dairy cows in herd that are local breeds</td>
</tr>
<tr>
<td>Exotic cows</td>
<td>Number of dairy cows in herd that are exotic breeds</td>
</tr>
<tr>
<td>Total liters per farmer</td>
<td>Total liters that a farmer brings to the collection center per day</td>
</tr>
<tr>
<td>Workers per farm</td>
<td>Number of individuals that work on the farm</td>
</tr>
<tr>
<td>Location of cows</td>
<td>Where the cows are kept and fed; pasture, free stall, tie stall, or combination</td>
</tr>
<tr>
<td>Milk sold</td>
<td>Whether or not the farmers sells milk to anyone other than the collection center</td>
</tr>
<tr>
<td>Milk kept for home cons.</td>
<td>Whether or not the farmers keep milk for their home consumption</td>
</tr>
<tr>
<td>Container</td>
<td>The material of the collection container; plastic or aluminum</td>
</tr>
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<td>Time b/t milking and CC</td>
<td>How long it takes the farmer to bring the milk to the collection center, &lt; 1 hour or &gt; 12 hrs</td>
</tr>
<tr>
<td>Closed/open</td>
<td>How the milk is stored during transport; closed or open container</td>
</tr>
<tr>
<td>Stored in cold water</td>
<td>How the milk collection container is stored prior to transport; in cold water or not</td>
</tr>
<tr>
<td>Often</td>
<td>How many times the farmer brings milk to the collection center per day; once or twice</td>
</tr>
<tr>
<td>Source of water</td>
<td>The source of water used for cleaning; tap, well, or river/stream</td>
</tr>
<tr>
<td>Hot water</td>
<td>Hot water is used to clean the collection containers; yes or no</td>
</tr>
<tr>
<td>Detergent</td>
<td>Detergent is used to clean the collection containers; yes or no</td>
</tr>
<tr>
<td>Pre Dip</td>
<td>Farmer cleans the teats prior to milking; yes or no</td>
</tr>
<tr>
<td>Dry</td>
<td>Farmer dries the teats after a pre-dip and prior to milking; yes or no</td>
</tr>
<tr>
<td>Post Dip</td>
<td>Farmer cleans the teats after milking; yes or no</td>
</tr>
<tr>
<td>Mastitis Check</td>
<td>Farmer checks for mastitis; yes or no</td>
</tr>
<tr>
<td>Antibiotic use</td>
<td>Farmer ever uses antibiotics to treat cows; yes or no</td>
</tr>
<tr>
<td>Vaccines</td>
<td>Vaccine is administered to the cows at least once a year; yes or no</td>
</tr>
<tr>
<td>Pre test</td>
<td>New cows are evaluated for disease prior to entering the herd; yes or no</td>
</tr>
<tr>
<td>Routine check ups</td>
<td>How often the farmer has cows evaluated for disease; &gt;1 per year, randomly, or never</td>
</tr>
<tr>
<td>Separate Workers</td>
<td>An individual is assigned only to milk; yes or no</td>
</tr>
<tr>
<td>Records</td>
<td>Records of any type are kept on the farm; yes or no</td>
</tr>
</tbody>
</table>
Appendix D: Figures

Figure 1: Map of Sampling Regions

A = Akaki, Addis Ababa, Ethiopia
B = Debre Zeit, Ethiopia
C = Selale, Oromia, Ethiopia
D = Asela, Oromia, Ethiopia
PFGE banding patterns with a similarity index >88.9% were grouped within the same genotypic cluster.