ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF VETERINARY MEDICINE

QUANTITATIVE RISK ASSESSMENT OF CONSUMING MILK CONTAMINATED WITH *STAPHYLOCOCCUS AUREUS* IN DEBRE-ZEIT

BY

FANTA DESISSA

JUNE 2010

DEBRE-ZEIT, ETHIOPIA

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A Thesis Submitted to the School of Veterinary Medicine, Addis Ababa University in Partial Fulfillment of the Requirement of the Degree of Master of Science in Tropical Public Health

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LIST OF ABBREVIATIONS

a_w	water activity
BAP:	Blood agar plates
BPW:	Buffer peptone water
CDC:	Centers for disease control and prevention
CFU:	Colony forming units
CI:	confidence interval
CNS:	Coagulase-negative *Staphylococcus*
CPS:	Coagulase-positive *Staphylococcus*
df:	Degree of freedom
DZ:	Debre-Zeit
EF:	Exfoliative toxins
ELFA:	Enzyme-linked fluorescent assay
ELISA:	Enzyme-linked immunosorbent and kits
FBD:	Food borne diseases
H_2O_2 :	Hydrogen peroxide
ISO:	International standard organization
K_2TeO_3	Potassium tellurite
KDa:	Kilo Dalton
MCC:	Milk collection center
MRSA:	Methicillin resistant *Staphylococcus aureus*
MSA:	Mannitol Salt Agar
NaCl:	Sodium chloride
NAP:	Nutrient agar plates
ng:	nanogram
PAB:	Purple agar base
PCR:	Polymerase chain reaction
PH:	power of hydrogen
PTSAgs:	Pyrogenic toxin superantigens
RRA:	Rapd Rural Appraisal

SEs:	Staphylococcal enterotoxins
SFP:	Staphylococcal Food Poisoning
SN:	Serial number
SPSS	Statistical Package for Social Sciences
SSSS:	Staphylococcal scalded skin syndrome
TSB:	Tryptone soya broth
TSS:	Toxic shock syndrome
TSST:	Toxic shock syndrome toxin
WHO:	World health organization
μg:	Microgram

ABSTRACT

A cross- sectional Study was conducted from October 2009 to March 2010 with the main objective to assess quantitatively the risk of consuming informally marketed milk contaminated with *Staphylococcus aureus* in Debre-zeit. The study employed a participatory risk assessment following the method recommended by the Codex Alimentarius Commission. Rapid rural appraisal and key informants interview with pre tested questionnaire were used to generate data on milk handling, quantity of milk produced and consumed and the habits of milk consumption. A deterministic model was developed to assess quantity of milk contaminated with *S. aureus* using data on prevalence of *S. aureus* in farm bulk milk and collection centers bulk milk, raw milk consumption habit, milk handling practices and quantity of milk at the point of exposures along the dairy value chain as risk inputs. Monte Carlo Simulation was used to simulate the quantity and proportion of milk contaminated with *Staphylococcus aureus* entero-toxin (SEs). Two hindered fifteen milk samples and two hundred eighteen participants were included in the study. The milk samples comprised raw farm bulk milk (170), milk collection centers bulk milk (25) and pasteurized milk (20). The participants included 170 dairy farmers, 14 milk collectors, 25 consumers, 1 production manager at processing plant and 8 hotel managers. The result showed 44 %, 72 % and 0% prevalence of *S. aureus* in farm bulk milk, milk collection centers bulk tank milk and pasteurized milk respectively. Comparison of the contamination rate of farm bulk milk with *S. aureus* among different collection centers showed statistically a significant differences(x^2=31.784, df=13, *p*=0.003). The milk produced and collected in peri-urban areas was significantly more contaminated with *S. aureus* (25/39, 64.1%) than milk produced and collected in urban areas (50/131, 38.2%) (x^2=7.18, df=1, *p*=0.007). About 24,000 L of milk was estimated to be produced per day in and around DZ. Of which, around 23% (5,500 L) milk was collected by Ada dairy cooperative society from the cooperative members. The survey result showed that 32% of the dairy producers and 36% of the consumers had the habit of consuming raw milk. The behavior of raw milk consumption was most common in illiterate group (62.9%) and the proportion was significantly higher than the other education level groups (p=0.0145). Risk factors for consumption of raw milk among dairy producers such as income, education level, location of farm and knowledge staphylococcal poisoning were considered. Among these, only residing in peri-urban areas was a risk factor for consuming raw milk (*p*<0.001). The study showed that 166.7 L (90%CI:

125.2-213.6), which is 0.7% (90% CI: 0.5-0.8) of the total daily production and everyday, 333 (90% CI: 250-427) people could acquire Staphylococcal poisoning in the urban areas of Debre-Zeit. Despite the limitations and the data gap, we demonstrated the benefit of participatory risk assessment not only as a risk evaluation tool but also as a helping device in the decision-making and the risk management.

Key words: Risk, deterministic model, Monte Carlo, Bulk milk, Dairy value chain, Debre-Zeit, *S. aureus*

1. INTRODUCTION

Food borne diseases or food poisonings are defined by the world health organization (WHO) as illnesses or diseases of infectious or toxic nature caused by the consumption of foods or water contaminated with bacteria and/or their toxins, parasites, viruses, or chemicals (Aycicek *et al*., 2005; Bania *et al*., 2006; Rho and Schaffner, 2007). A food borne disease (FBD) outbreak is said to occur if similar illness, often gastrointestinal, in a minimum of two people and evidence of food as the sources are confirmed (Loir *et al.,* 2003; Shah, 2003). In many countries, national health care organizations defined FBD outbreaks as the occurrence of two or more cases of a similar illnesses resulting from the ingestion of a common food (Loir *et al*., 2003; Baron, 2007)**.**

One organism of particular interest to food safety is *Staphylococcus aureus*. This facultative anaerobic gram positive bacterium is a major cause of food borne intoxications and outbreaks throughout the world because of its ubiquity and its ability to persist and grow under various conditions. *S. aureus* is able to survive and multiply in a variety of food substrates, at a variety of temperatures (7-48 ^{o} C) and pH values (4.5-9.3) and at water activities of (0.83 to 0.99) (Shah, 2003).

Unlike other common food borne illnesses that require consumption of, and infection by, viable pathogenic microbial cells, sickness associated with *S. aureus* occurs as a result of ingestion of numerous heat- and protease-stable staphylococcal enterotoxins (SEs) produced under specific environmental conditions when the population density of the pathogen reaches 10^5 CFU/ml. This bacterial load allows the production of 20ng to 1μg of SE sufficient to determine symptoms of staphylococcal food poisoning (SFP) in human beings (Shah, 2003; Lourdes *et al*., 2004; Todar, 2008)*.*

The public health hazard due to ingestion of foods contaminated with *S. aureus* is particularly linked to the ability of 50% of these strains to produce thermo-stable SEs associated with food poisoning (Aycicek *et al*., 2005; Kerouanton *et al*., 2007).

Staphylococcus aureus can cause SFP by ingestion of preformed toxin with an incubation period of 1-6 hours as well as by infecting both local tissues and the systemic circulation with variable and indefinite incubation period, most commonly 4-10 days. Patients become symptomatic after ingestion of thermo-stable SEs at an approximate dose of 0.1 to 1.0 mg/kg of body weight and SFP caused by ingestion of this SEs have a rapid onset (Miwa *et al*., 2001; Chiang *et al*., 2008).

Staphylococcal food poisoning is characterized by, nausea, vomiting, diarrhea, sweating, abdominal cramping, and prostration in human beings (Jay, 2000; Acco *et al*., 2003). The duration of illness typically is 1 to 2 days. However, it usually takes three days to recovery completely and sometimes longer in severe cases (Jay, 2000; Aycicek *et al*., 2005).

The short incubation period, brevity of illness, and usual lack of fever help to distinguish SFP from other types of food poisoning (Omoe *et al*., 2005; Chiang *et al*., 2008.Previous reviews have found hospitalization rates of staphylococcal intoxication as high as 14%. Although not considered especially lethal, death can ensue if large amounts of SE are ingested; fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive individuals such as immunocompromised, elderly and children (Atanassova *et al*., 2001; Aycicek *et al*., 2005; Kerouanton *et al*., 2007; Rho and Schaffner, 2007; Walderhaug, 2007*)*.

Many foods will support growth of *S. aureus* and toxin production; however milk, dairy products and meats, especially handled foods, are common vehicles and are probably the most frequently implicated which play an important role in SFP (Jay, 2000; Smith, 2007).

Milk and dairy products are implicated as the sources of illness associated with milk collection and normal processing conditions that may allow the presence of bacteria in the dairy cows and the dairy environment to be introduced directly into the milk. Once introduced, the highly nutritive milk medium supports rapid microbial growth. Consequently, the potential for food borne illness and intoxication from consumption of milk and dairy products is of concern (Halpin-Dohnalek *et al*., 1989; Fujikawa and Morozumi, 2006).

Although some outbreaks have been associated with pasteurized milk, pasteurization is still considered an extremely effective method for reducing bacterial pathogens in milk, and these

outbreak events usually are rare (Cohen, 2000). However, not all milk and dairy products are pasteurized, and raw unpasteurized milk is widely consumed throughout the world (Schmidt and Davidson, 2008).

 Raw milk has been reported to be a known vehicle for pathogens for more than 100 years. Outbreaks associated with the consumption of raw milk occur routinely. Consumption of raw milk is a high-risk behavior and will continue to cause morbidity and mortality until people stop it (Keene, 1999; Gillespie *et al*., 2003).

Similarly, majority of Ethiopian population consume raw milk and raw milk products including cheese, cream, butter and yoghurt (Ashenafi, 1990; Ashenafi and Beyene, 1994). Besides informal marketing of raw milk in and around Debre-Zeit is very common (Abera, 2008). As a result, the possibility of incidence of SFP due to the consumption of dairy products is common in our country (Ashenafi and Beyene, 1994; Yilma *et al*., 2007).

The safety of raw milk and raw milk products with respect to staphylococcal poisoning is of great concern around the world (De Buyser *et al.,* 2001). This is especially true in developing countries like Ethiopia where production and consumption of raw milk and various dairy products often takes place under unsatisfactory hygiene conditions (Ashenafi, 1990; Wubete, 2004).

Hence, to assess the safety of raw milk, risk assessment, tool for control of biological hazards in foods, is of essential (CAC, 2007).

So far, there have been no studies with regarding to the quantitative risk assessment of SFP that might attributed to consumption of milk and milk products in the Debre-Zeit area. Therefore, the objectives of the present study include:

General objective**:**
- To assess the risk of consuming informally marketed milk contaminated with *Staphylococcus aureus* in Debre-zeit

Specific objectives:
- To investigate the existing dairy value chain in and around Debre-Zeit

- To assess the milk handling and raw milk consumption practices along the dairy value chain

- To assess risk factors associated with consumption of raw milk

- To determine the prevalence of *S. aureus* in informally marketed milk at farm and milk collection centers

- To estimate the probability or proportion of milk contaminated with *S. aureus,* SEs and the associated number of illness.

Hypothesis

- Informally marketed milk in DZ is free of *S. aureus,* the probability of consuming milk contaminated with *S. aureus* through informal market chain, and risk to human is insignificant.

2. LITERATURE REVIEW

2.1. Overview of food safety

Food borne diseases or food poisonings are defined by WHO as an illness or diseases of infectious or toxic nature caused by the consumption of foods or water contaminated with bacteria and/or their toxins, parasites, viruses, or chemicals (Aycicek *et al*., 2005; Bania *et al*., 2006; Rho and Schaffner, 2007).

Food-borne diseases remain a real and formidable problem in both developed and developing countries, causing great human suffering and significant economic losses. Up to one third of the population of developed countries may be affected by food-borne diseases each year, and the problem is likely to be even more widespread in developing countries, where food and water-borne diarrhoeal diseases kill an estimated 2.2 million people each year, most of them Children (FAO/WHO, 2006).

Food safety is therefore a fundamental public health concern, and achieving a safe food supply poses major challenges for national food safety officials. Changing global patterns of food production, international trade, technology, public expectations for health protection and many other factors have created an increasingly demanding environment in which food safety systems operate. An array of food-borne hazards, both familiar and new, pose risks to health and obstacles to international trade in foods. These risks must be assessed and managed to meet growing and increasingly complex sets of national objectives (CAC, 2007).

A food-borne hazard is defined by Codex as "a biological, chemical or physical agent in, or condition of, food, with the potential to cause an adverse health effect. Many of these hazards have long been recognized and addressed by food safety controls, however, some of the changing global conditions may have exacerbated the problems they pose (CAC, 1999).

2.2. Microbial food Risk analysis

Risk is defined as a combination of the probability and the consequences of a hazard. A risk in the context of food safety is the probability and the consequences of adverse health effects following the ingestion of food. The separation of risk into two components is useful, since risk may be managed both by actions to reduce the probability and the consequences of the adverse event. The second component is often overlooked in microbial risk assessments, although it may implicitly be considered in the selection of the biological end- point in the dose-response relationship, e.g. diarrhea, morbidity, mortality (CAC,2007).

Risk analysis is the systematic use of available information to identify possible sources of harm, assess their likelihood of occurrence and impact, and implement methods to avoid or reduce them (CAC,2007). Originally applied to engineering and actuarial problems, now it is considered best practice for food safety management. In the context of food safety, it is a tool, which in a formalized, systematic and transparent way, enables responsible authorities and international organizations to understand and if necessary evaluate options to reduce a health risk. As such, risk analysis complements other tools such as Good Manufacturing Practice and HACCP (CAC, 1999; Grace *et al*., 2007).

Conventional food safety studies typically determine the prevalence of disease-causing pathogens in marketed food. This tends to be widely reported by the media and results in panic among consumers and policymakers alike. The responses are dramatic drops in consumption, adversely affecting the livelihoods of farmers and traders and the nutrition of consumers. Risk-based food safety approaches shift the focus from hazards (sources of harm) to risk. As such, they would better address the concerns of consumers and decision makers, who are more interested in the impacts of pathogens on human health than in their presence, and who are concerned about farmers' livelihoods and economic impacts as well as health impacts. Knowledge of impacts is also much more useful in deciding resource allocation for management of hazards and appropriate levels of protection. This is particularly important in informally marketed food in developing countries where animal source foods are believed to be a major contributor to the overall disease burden (Grace *et al*., 2007).

Effective management of risks arising from microbial hazards is technically complex. Food safety has been traditionally, and will continue to be, the responsibility of industry operating an array of control measures relating to the food hygiene within an overall regulatory framework. Recently, risk analysis, involving its component parts of risk assessment, risk management and risk communication, has been introduced as a new approach in evaluating and controlling microbial hazards to help protecting the health of consumers and ensure fair practices in food trade. It could also facilitate the judgment of equivalence of food safety control systems (CAC, 1999; CAC, 2007).

2.2.1. Risk assessment

Risk assessment is a science-based process in which questions that have been formulated during the risk evaluation step of the risk management process are addressed to develop an understanding of the problem and to come up with risk estimates. It involves four consecutive steps: hazard identification and exposure assessment (Lam-merding and Fazil, 2000) and hazard characterization and risk characterization (Buchanan *et al.*, 2000).

Hazard identification

Hazard identification s the identification of biological, chemical and physical agents capable of causing adverse health effects that may be present in a particular food or group of foods (CAC, 999). For microbial agents, the purpose of hazard identification is to identify the microorganisms or the microbial toxins of concern with food. Hazard identification will predominately be a qualitative process. Information on hazards can be obtained from scientific literature, clinical studies, epidemiological studies and surveillance, laboratory animal studies, investigations of the characteristics of microorganisms, the interaction between microorganisms and their environment through the food chain from primary production up to consumption, and studies on analogous microorganisms. Also obtained from databases such as those in the food industry, government agencies, and relevant international organizations and through solicitation of opinions of experts (Lammerding and Fazil, 2000; FAO/WHO, 2000; FAO/WHO, 2006).

Exposure assessment

Exposure Assessment includes an assessment of the extent of actual or anticipated human exposure. For microbiological agents, Exposure Assessments might be based on the potential extent of food contamination by a particular agent or its toxins, and on dietary information. Exposure assessment should specify the unit of food that is of interest, i.e., the portion size in most/all cases of acute illness (FAO/WHO, 2006).

Factors that must be considered for exposure assessment include the frequency of contamination of foods by the pathogenic agent and its level in those foods over time. These are further influenced, by the characteristics of the pathogenic agent, the microbiological ecology of the food, the initial contamination of the raw material including considerations of regional differences and seasonality of production, the level of sanitation and process controls, the methods of processing, packaging, distribution and storage of the foods, as well as any preparation steps such as cooking and holding (CAC, 2007).

Another factor is patterns of consumption. This relates to socio-economic and cultural backgrounds, ethnicity, seasonality, age differences (population demographics), regional differences, and consumer preferences and behavior (FAO/WHO, 2006).

Other factors to be considered include the role of the food handler as a source of contamination, the amount of hand contact with the product, and the potential impact of abusive environmental time/temperature relationships. Microbial pathogen levels can be dynamic and while they may be kept low, for example, by proper time/temperature controls during food processing, they can substantially increase with abuse conditions (for example, improper food storage temperatures or cross contamination from other foods) (FAO/WHO, 2003).

Exposure Assessment estimates the level, within various levels of uncertainty, of microbiological pathogens or microbiological toxins, and the likelihood of their occurrence in foods at the time of consumption. Qualitatively foods can be categorized according to the likelihood that the foodstuff will or will not be contaminated at its source; whether or not the food can support the growth of the

pathogen of concern; whether there is substantial potential for abusive handling of the food; or whether the food will be subjected to a heat process(CAC,2007).

The presence, growth, survival, or death of microorganisms, including pathogens in foods, are influenced by processing and packaging, the storage environment, including the temperature of storage, the relative humidity of the environment, and the gaseous composition of the atmosphere. Other relevant factors include pH, moisture content or water activity (aw), nutrient content, the presence of antimicrobial substances, and competing microflora. Predictive microbiology can be a useful tool in an Exposure Assessment (CAC, 1999; FAO/WHO, 2000; Lammerding and Fazil, 2000).

Therefore, the Exposure Assessment should describe the pathway from production to consumption. Scenarios can be constructed to predict the range of possible exposures. The scenarios might reflect effects of processing, such as hygienic design, cleaning and disinfection, as well as the time/temperature and other conditions of the food history, food handling and consumption patterns, regulatory controls, and surveillance systems (FAO/WHO, 2006).

Hazard characterization

This step provides a qualitative or quantitative description of the severity and duration of adverse effects that may result from the ingestion of a microorganism or its toxin in food. A desirable feature of Hazard Characterization is ideally establishing a dose-response relationship by taking the different end points such as infection or illness into consideration. In the absence of a known dose-response relationship, risk assessment tools such as expert elicitations could be used to consider various factors, such as infectivity, necessary to describe hazard characterizations. Additionally, experts may be able to devise ranking systems so that they can be used to characterize severity and/or duration of disease (Buchanan *et al*., 2000; FAO/WHO, 2003; FAO/WHO, 2006).

Furthermore, several important factors need to be considered in hazard characterization. These are related to both the microorganism, and the human host. In relation to the microorganism, the

following are important: microorganisms are capable of replicating; the virulence and infectivity of microorganisms can change depending on their interaction with the host and the environment; genetic material can be transferred between microorganisms leading to the transfer of characteristics such as antibiotic resistance and virulence factors; microorganisms can be spread through secondary and tertiary transmission; the onset of clinical symptoms can be substantially delayed following exposure; microorganisms can persist in certain individuals leading to continued excretion of the microorganism and continued risk of spread of infection; low doses of some microorganisms can in some cases cause a severe effect; and the attributes of a food that may alter the microbial pathogenicity, e.g., high fat content of a food vehicle (FAO/WHO, 2006;CAC, 2007).

In relation to the host, the following may be important: genetic factors such as Human Leucocyte Antigen (HLA) type; increased susceptibility due to breakdowns of physiological barriers; individual host susceptibility characteristics such as age, pregnancy, nutrition, health and medication status, concurrent infections, immune status and previous exposure history; population characteristics such as population immunity, access to and use of medical care, and persistence of the organism in the population (Buchanan *et al*., 2000; FAO/WHO, 2006).

Risk characterization

It is the interactive exchange of information and opinions concerning risk and risk management among risk assessors, risk mangers, consumers and other interested parties (CAC,1999). Risk Characterization represents the integration of the hazard identification, hazard characterization, and exposure assessment determinations to obtain a risk Estimate; providing a qualitative or quantitative estimate of the likelihood and severity of the adverse effects that could occur in a given population, including a description of the uncertainties associated with these estimates (FAO/WHO, 2000). These estimates can be assessed by comparison with independent epidemiological data that relate hazards to disease prevalence. Risk characterization brings together all of the qualitative or quantitative information of the previous steps to provide a soundly based estimate of risk for a given population. Risk characterization depends on available data and

expert judgments. The weight of evidence integrating quantitative and qualitative data may permit only a qualitative estimate of risk (CAC, 1999; FAO/WHO, 2000; FAO/WHO, 2006).

The degree of confidence in the final estimation of risk will depend on the variability, uncertainty, and assumptions identified in all previous steps. Differentiation of uncertainty and variability is important in subsequent selections of risk management options. Uncertainty is associated with the data themselves, and with the choice of model. Data uncertainties include those that might arise in the evaluation and extrapolation of information obtained from epidemiological, microbiological, and laboratory animal studies. Uncertainties arise whenever attempts are made to use data concerning the occurrence of certain phenomena obtained under one set of conditions to make estimations or predictions about phenomena likely to occur under other sets of conditions for which data are not available. Biological variation includes the differences in virulence that exist in microbiological populations and variability in susceptibility within the human population and particular subpopulations. It is important to demonstrate the influence of the estimates and assumptions used in Risk Assessment; for quantitative Risk Assessment this can be done using sensitivity and uncertainty analyses (CAC, 1999; Buchanan *et al*., 2000; FAO/WHO, 2000).

2.2.3. Risk Management

It consists of identifying, evaluating, selecting and implementing specific management measures to mitigate risks potential. The risk analyst identifies risk and may counsel alternatives. Decision on preventive measures belongs to the public health policy maker and politicians in local state or national government (FAO/WHO, 1997; FAO/WHO, 2006).

2.2.4. Risk communication

The purpose of risk communication is to translate scientific information into messages that help the public put risks into perspective and make decision about risks. Successful risk communication means that the message is understood by the target audience. Risk-based approaches brought new insights and are now standard for food safety issues in developed countries as well as being the basis of rules governing international trade in food products (FAO/WHO, 1998; FAO/WHO, 2006).

2.3. Overview of the dairy sector in Ethiopia

Over the last decade, the dairy sector in Ethiopia has shown considerable progress. Total milk production grew at an estimated rate of 3% as compared to 1.63-1.66% during the period of 1975-1992, thus ending the long-time trend of declining per capita milk production in the country (Ahmed *et al.,* 2003). The progress achieved is mainly due to technological intervention, policy reforms and population growth. According to Kelay (2002) and Ahmed *et al.* (2003) the dairy sector in Ethiopia is expected to continue to grow over the next one to two decades. The large potential for dairy development in the country, the expected growth of income of the population, increased urbanization and improved policy environment were the ones considered for the above indicated expectation. The shift towards market economy is creating large opportunity for private investment in urban and peri-urban dairying. However, the main source of growth is expected to be the growth in demand for dairy products (Ahmed *et al.,* 2003).

Given the considerable potential for improving smallholder income and employment generation from high-value dairy products (Staal *et al.,* 2001), development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country. Ethiopia with an average annual per capita income of less than $100 is among the poorest countries in sub-Saharan African (FAO, 2001). According to FAO (2001), there is high level of malnutrition with an estimation of about 51% of the population being undernourished and over two million people being chronically food unsecured.

2.3.1. Dairy production system in Ethiopia

Three major systems of dairy production can be distinguished in Ethiopia. These are lowland pastoral dairy production systems, rural highland smallholder dairy production system, urban and peri-urban dairy production system (Kelay, 2002).

Lowland pastoralists dairy farming

About 30% of the livestock population in Ethiopia is found in the pastoral areas. These areas comprise 50% of the total land area of the country and have altitudes below 1500 m a.s.l. Pastoralism is the major dairy production system in the lowland. Livestock does not provide inputs

for crop production but they are the backbone of household economies by providing all of the consumable and saleable out puts and regarded as insurance against adversity. Milk production is dependent on season due to the rainfall pattern that influences feed availability (Ketema and Tsehay, 1995).

Rural high land smallholder dairy farming

There are two types of systems in the highland: the traditional system that is based on indigenous breeds and market-oriented system that is based on crossbred dairy cattle (Redda, 2001). The average lactation yield for indigenous cows is 524 liters for 239 days and the average age at first calving is 53 months and the average calving interval is 25 months. The average milk yield and lactation length for crossbred cows ranges from 518-1448kg and 110-300 days, respectively, depending on the breed type. The household mainly consumes the milk produced in the traditional system while most of the milk is sold to generate income in the market-oriented system (Tesfaye, 1995).

Urban and peri-urban dairy farming

It includes small and large private and state farms in urban and peri-urban areas concentrated in the central highland plateaus (Felleke and Geda, 2001). This sector is commercial and mainly based on the use of grade and crossbred animals that have the potential to produce 1120-2500 liters over 279-day lactation. This production system is now expanding in the highlands among mixed crop-livestock farmers such as those found in Selale and Holetta areas, and serves as the major milk supplier to the urban market (Gebrewold *et al.,* 2000; Holloway *et al.,* 2000).

2.3.2. The role of the dairy sector in the Ethiopian economy

At household level, dairying is important in one way or another in all the farming systems of Ethiopia. In pastoralist and the mixed crop-livestock farming system milk is the most important source of protein (Tilahun, 1995). In the mixed crop-livestock farming system milk is also used mainly as food to the household and to a lesser extent as a source of income (FAO, 1999). In urban and peri-urban areas, dairy production is practiced mainly as a source of income. Ethiopians

consume less dairy products (as per capita consumption is 17kg per head) compared to the average 26 kg per head for Africa (Gebrewold *et al.,* 2000).

Dairy animals as source of *S. aureus* contamination

Dairy animals are probably the main source of contamination of raw milk with *S. aureus*. In particular, dairy animals with subclinical *S. aureus* mastitis may shed large numbers of *S. aureus* into the milk. However, contamination of raw milk and raw milk products from human handling or from the environment during manufacture is also possible (Jorgensen *et al.,* 2005).

2.4. Staphylococcal infection and food poisoning

2.4.1. Etiology

Staphylococcal food poisoning is a food borne poisoning attributed to ingestion of contaminated food in which the enterotoxigenic strains of staphylococcus can multiply reaching about 10^5 CFU/g of food. *Staphylococcus aureus* is a major cause of food borne intoxication and outbreaks throughout the world because of its ubiquity and its ability to persist and grow under various conditions (Salandra *et al.*, 2008).

General characteristics

Staphylococcus aureus is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. The golden appearance is the etymological root of the bacteria's name; aureus means "golden" in Latin (Freeman, 1985; Quinn *et al.*, 1999; Silva *et al.*, 2000).

The bacterium multiply by simple division into two, and under suitable conditions of environment and temperature, this occurs every 15-30 minutes. Thus, one cell could become over 2 million in 7 hours and 7000 million cells after 12 hours continuous growth (Quinn *et al.*, 1999; Jay, 2000).

Staphylococcus aureus is catalase positive and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish from enterococci and streptococci.

A small percentage of *S. aureus* can be differentiated from most other staphylococci by the coagulase test: *S. aureus* is primarily coagulase-positive (meaning that it can produce "coagulase", a protein product, which is an enzyme) that causes clot formation while most other *Staphylococcus* species are coagulase-negative (Freeman, 1985; Silva *et al*., 2000).

The bacterium do not produce endospores but are highly resistant to high osmotic conditions and desiccation, especially when associated with organic matter such as blood, pus, and other tissue fluids. These properties facilitate its survival in the environment, growth in food, and communicability. However, usually it is readily killed at cooking, pasteurization temperatures; but survives frozen storage. Heat resistance of *S. aureus* is increased in dry, high-fat and high-salt foods. On the other hand, SEs are extremely resistant to heat (Quinn *et al*., 1999; Silva *et al*., 2000; Ash, 2008).

Although *S. aureus* is commonly found on the skin of a wide variety of mammals and birds and on the environment, humans are thought to be the primary source of strains associated with food matrix staphylococcal intoxication (Salyers and Whitt, 2002; Sandel and McKillip, 2004).

Taxonomy and classification

The name *Staphylococcus* (staphyle= bunch of grapes in Greece) was introduced in 1883 by Ogston. One year later, Rosenbach used the term in a taxonomic sense and provided the first description of the genus *Staphylococcus* (Todar, 2008). Taxonomically, *S. aureus* is in the Bacterial family of staphylococcaceae, genus *Staphylococcus*. The scientific classification of *Staphylococcus* according to Shah, 2003 and Todar, 2008 is as follows:

Kingdom: Bacteria   Order: Bacillales

Phylum: Firmicutes   Family: staphylococcaceae

Class: Bacilli   Genus: Staphylococcus

Species: *aureus*

Morphology

The staphylococcus has a gram-positive cell composition, with a unique peptidoglycan structure that is highly cross linked with bridges of amino acids (Foster, 1991; Shah, 2003; Hein *et al*., 2005). In addition to the usual peptidoglycan (murein), it has two special components in the cell wall: Protein A and Teichoic acids-polyribitol glycerophosphates that are unique to staphylococci. Protein A is linked to the peptidoglycan with an outer end that binds to the Fc receptor of IgG, protecting the microbe from phagocytosis (opsonisation) whereas Teichoic acids-polyribitol glycerophosphates are involved in complement activation and attachment to mucosal surfaces as they bind to fibronectin (Smith, 2007; Walderhaug, 2007; Todar, 2008).

The bacterium cells are spherical (cocci),which tend to be arranged in pairs, short chains, or typically occurring in bunched, grape-like irregular clusters when viewed through a microscope, owing to cell division in multiple planes (Jay, 2000; Shah, 2003). The most obvious morphological characteristic is its marked tendency to occur as masses of cells in grape like clusters. This happens because of the geometry (divide in two planes) (Freeman, 1985).

In the genus *Staphylococcus, S. aureus* has large, round, golden-yellow colonies, often with haemolysis, when grown on blood agar plates. Some strains of *S. aureus* are capable of producing *staphyloxanthin* - a carotenoid pigment that acts as a virulence factor. This pigment has an antioxidant action that helps the microbe to evade killing with reactive oxygen used by the host immune system. It is thought that staphyloxantin is responsible for *S. aureus* characteristic golden color (Tsegmed, 2006; Todar, 2008).

Growth requirements

The *S. aureus* grows in the temperature range of $7\,^{o}C$ to $48\,^{o}C$ and produce enterotoxin from $10\,^{o}C$ - $48\,^{o}C$, with optimum temperature for growth is $35\,^{o}C$ - $37\,^{o}C$ and optimum enterotoxin production at $40\,^{0}C$ - $45\,^{o}C$ (Aycicek *et al*., 2005; Ash, 2008).

Growth and toxin production of *Staphylococcus aureus* is best in the presence of oxygen but can grow anaerobically. It is not regarded as a good competitor with other bacteria. Although growth usually is constrained by the presence of competing organisms, *S. aureus* thrives in environments relatively free of competition from other bacteria, such as foods with high concentrations of salt and sugar that impede the growth of other organisms (Silva *et al*., 2000; Aycicek *et al*., 2005; Ash, 2008).

Staphylococcus aureus is facultative anaerobe that grows best by aerobic respiration or fermentation that yields principally lactic acid (Freeman, 1985; Silva *et al*., 2000). The optimum pH for growth is 7 - 7.5 with minimum pH of 4.2 and maximum pH of 9.3. Therefore, foods with a pH around 7 are ideal for the growth and most animal food products including meat, fish, poultry, eggs, and milk have been reported to be best media for growth of the microorganism (Rho and Schaffner, 2007).

The low water activity (a_w) at which *Staphylococcus aureus* grows is of particularly significant. The bacterium is resistant to drying and may grows and produces enterotoxins in foods with a_w as low as 0.85. They can grow in up to 25% NaCl but grows well in 7 -10% NaCl. The optimum a_w for growth is 0.99. Its ability to grow at low a_w means that it has a competitive advantage on low a_w foods (Hocking and Doyle, 1997; Aycicek *et al*., 2005; Ash, 2008).

The optimum pH for toxin production is 5.3-7.0 (range 4.8- 9.0), a_w is 0.90 (range 0.86 - 0.99) and greatest toxin production is in the presence of oxygen. Combinations of different inhibitory factors such as NaCl content and pH can be used to control toxin production and growth of *S. aureus* (Morandi *et al*., 2007; Walderhaug, 2007).

It is not highly fastidious in its nutritive requirements and grows readily on the usual meat extract peptone mediums. Growth is most profuse on sheep blood agar mediums commonly used for isolation of the pathogenic forms (Freeman, 1985; Quinn *et al*., 1999; Jay, 2000).

Virulence factors

The importance of *Staphylococcus aureus* to both the clinical and food settings is associated to the wide variety of specific virulence determinants (Acco *et al*., 2003; Sandel and McKillip, 2004). It expresses a variety of extra cellular proteins and polysaccharides, which correlated with virulence. Virulence results from the combined effect of many factors expressed during infection (Foster, 1991; Shah, 2003; Todar, 2008).

The major potential virulence factors of *Staphylococcus aureus* include surface proteins that promote colonization of host tissues, invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase), surface factors that inhibit phagocytic engulfment (capsule, immunoglobulin binding protein A) and biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production). Further more, Immunological disguises (protein A, coagulase, clotting factor), membrane-damaging toxins that lyses eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin) and exotoxins that damage host tissues or otherwise provoke symptoms of disease and Inherent and acquired resistance to antimicrobial agents (Foster, 1991; Todar, 2008).

In addition to genetic information on the chromosome, pathogenic *Staphylococcus aureus* often contain accessory elements such as plasmids, bacteriophages, pathogenicity islands (DNA clusters containing genes associated with pathogenesis) and transposons. These elements harbor genes that encode toxins or resistance to antimicrobial agents and may be transferred to other strains (Martın *et al*., 2004). Genes involved in virulence, especially those coding for exotoxins and surface-binding proteins, are coordinately or simultaneously regulated by loci on the chromosome (Foster, 1991; Rowland *et al*., 1994; Shah, 2003).

Depending on the strain, *S. aureus* is capable of secreting several toxins, which can be categorized into three groups: pyrogenic toxin superantigens (PTSAgs), exfoliative toxins (EF) and other toxins. Many of these toxins are associated with specific diseases (Loir *et al.,* 2003; Sandel and McKillip, 2004).

The first group comprising PTSAgs has super antigen activities that induce toxic shock syndrome (TSS). This group includes the toxin TSST-1, which causes TSS associated with tampon use. The staphylococcal enterotoxins, which cause a form of food poisoning, are included in this group (Loir *et al*., 2003).

Exfoliative toxins (EFs) are implicated in the disease staphylococcal scalded-skin syndrome (SSSS), which occurs most commonly in infants and young children. It also may occur as epidemics in hospital nurseries. The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS (Martın *et al*., 2004).

Other staphylococcal toxins that act on cell membranes include alpha-toxin, beta-toxin, delta-toxin, and several bicomponent toxins. The bicomponent toxin Panton-Valentine leukocidin (PVL) is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community-associated MRSA strains (Zhu *et al,* 2008).

Protein A is a protein that is anchored to staphylococcal peptidoglycan pentaglycine bridges by the transpeptidase Sortase A (Schneewind *et al*., 1995). Protein A is an IgG-binding protein that binds to the Fc region of an antibody. In fact, studies involving mutation of genes coding for Protein A resulted in a lowered virulence of *S. aureus* as measured by survival in blood, and this has led to speculation that Protein A contributed virulence requires binding of antibody Fc regions (Patel *et al*., 1987). Protein A in various recombinant forms has been used for decades to bind and purify a wide range of antibodies by immunoaffinity chromatography. Transpeptidases such as the sortases that are responsible for anchoring factors like Protein A to the staphylococcal peptidoglycan are being studied in hopes of developing new antibiotics to target MRSA infections (Zhu *et al,* 2008).

2.4.2. The staphylococcal enterotoxins and food poisoning

Staphylococcal enterotoxins are exoproteins produced in food and ingested by humans give rise to symptoms of acute gastroenteritis (responsible for SFP). The toxins have been shown to be proteins of low molecular weight, approximately 27–31 kDa, consisting only of amino acids and are usually produced by CPS species (Ash, 2008; Chiang *et al*., 2008).

The SEs are short proteins belonging to a large family of pyrogenic toxin super antigens encoded by phage, chromosome or plasmid genes with a disulphide bridge secreted in the medium and soluble in water and saline solutions. They are rich in lysine, aspartic acid, glutamic acid, and tyrosine residues. Most of them possess a cystine loop required for proper conformation and which is probably involved in the emetic activity (Loir *et al*., 2003; Salandra *et al*., 2008).

Staphylococcal enterotoxins are highly stable, resist most proteolytic enzymes, such as pepsin, or trypsin, and thus keep their activity in the digestive tract after ingestion. They are highly heat resistant as well, which can resist $100\ ^{o}$C for at least 30 minutes and probably longer. Although pasteurization and cooking kills staphylococci cells which are heat labile, thermo-stable SEs generally retain their biological activity. Thus, cases of illness might occur although no viable bacteria can be isolated from the suspected foodstuff and since SEs are more heat stable than the staphylococci bacteria, it is possible to test a food product and obtain negative staphylococci culture results and positive SEs tests (Atanassova *et al*., 2001; Soejima *et al*., 2007).

The amount of enterotoxins produced is determined by factors such as the composition of the food, competition from other microorganisms (the presence of other bacteria affects the production of enterotoxin apparently by limiting the multiplication of the staphylococci), temperature and time (Hagstad and Hubbert, 1986; Salyers and Whitt, 2002).

A family of 14 different SE types has been identified, which share structure and sequence similarities, of which the antigenic types (named SE-A, B, C, D, E) are most commonly encountered in SFP (Kerouanton *et al*., 2007). In general, SE-A is recovered from food poisoning outbreaks more often than any of the others, with SE-D being second most frequent and the fewest number of outbreaks are associated with SE-E (Jay, 2000; Shah, 2003).

Recently, additional SEs have been identified: SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, and SEU. Many of these newly discovered enterotoxins are structurally similar to the classic enterotoxins, which suggest that they also may illicit foodborne illness when consumed

in large enough doses. The significance of these SEs in causing foodborne intoxication remains largely unknown and requires both future research and increased surveillance (Rall *et al*., 2008).

The toxins act on the emetic receptors on the abdominal viscera causing stimulation of the emetic center of the brain via vagus and sympathetic nerves. The nerve stimulation ultimately results in causing diarrhoea and vomiting (Atanassova *et al*., 2001; Walderhaug, 2007).

When SEs are expressed systematically, they mediate two illnesses, TSS and SSSS. In both diseases, exotoxins are produced during an infection, diffuse from the site of infection, and are carried by the blood (toxemia) to other sites of the body, causing symptoms to develop at sites distant from the infection. Toxic shock syndrome toxin is produced when SEs are expressed systemically and it is the cause of TSS. It is very weakly related to enterotoxins and does not have emetic activity (Bania, 2006; Smith, 2007). Toxic shock syndrome is an acute life-threatening illness mediated by staphylococcal superantigen exotoxins and can occur as a sequel to any staphylococcal infection if an enterotoxin or TSST is released systemically and the host lacks appropriate neutralizing antibodies (Foster, 1991; Salyers and Whitt, 2002). Staphylococcal scalded skin syndrome, also known as Ritter's disease characterized by dermatologic abnormalities (Shah, 2003; Todar, 2008).

Because of the importance of these toxins in the public health and food sectors, an efficient screening to detect the prevalence of enterotoxic strains in foods is required. Indeed, not all staphylococci produce SEs, and SEs production may be insufficient for food intoxication (Martın *et al*., 2004; Turutoglu *et al*., 2005; Morandi *et al*., 2007*)*.

2.4.3. Pathogenesis

Staphylococcus aureus is an important pathogen due to a combination of toxin mediated virulence and invasiveness. The toxins liberated by the organism may have effects at sites distant from the focus of infection or colonization (Foster, 1991; Loir *et al*., 2003; Soejima *et al*., 2007).

Tissue invasion

The event that leads to infection is initiated with carriage of the organism. Then the organism disseminated via hand carriage to body sites where infection may occur (either through overt breaks in dermal surfaces, such as vascular catheterization or operative incisions, or through less evident breakdown in barrier function, such as eczema or shaving associated trauma). The hallmark of staphylococcal infection is the abscess, which consists of a fibrin wall surrounded by inflamed tissues enclosing a central core of pus containing organisms and leukocytes. From this focus of infection, the organisms may be disseminated hematogenously, even from the smallest abscess (Loir *et al*., 2003; Smith, 2007).
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The ability to elaborate proteolytic enzymes facilitates the process. This may result in pneumonia, bone and joint infection, and infection of the heart valves. In immunocompromised hosts (eg, patients with cancer who are neutropenic and have a central venous line), 2030% develop serious complications or fatal sepsis following catheter related *S aureus* bacteremia. Persistent deep-seated infections have now been linked to small colony variants of the organism. This population is more resistant to antibiotics and grows slowly (Soejima *et al*., 2007; Todar, 2008).

Toxin mediated diseases

 Staphylococcus aureus also elaborates toxins that can cause specific diseases or syndromes. Enterotoxin producing strains of *S aureus* cause one of the most common food borne illnesses by preformed toxin production with an incubation period of 1-6 hours; as well as by infecting both local tissues and the systemic circulation with variable and indefinite incubation period, most commonly 4-10 days. Enterotoxins are low-molecular-weight extracellular, superantigenic chemicals that initiate nonspecific T-cell proliferation resulting in severe acute onset of watery diarrhea, nausea, vomiting, and abdominal pain (Baron, 2007; Chiang *et al.,* 2008).

A rare but well described disorder in neonates and young children is staphylococcal scalded skin syndrome (Ritter disease). The organism produces an exfoliative toxin produced by strains belonging to phage group II. Initial features include fever, erythema, and blisters, which eventually

rupture and leave a red base. Gentle shearing forces on intact skin cause the upper epidermis to slip at a plane of cleavage in the skin, which is known as the Nikolsky sign ((Loir *et al*., 2003; Smith, 2007).

The most feared manifestation of *S aureus* toxin production is toxic shock syndrome (TSS). Although first described in children, it was most frequently associated with women using tampons during menstruation. Since the early 1990s, at least half of the cases have not been associated with menstruation. The syndrome is associated with strains that produce the exotoxin TSST1, but strains that produce enterotoxin B and enterotoxin C may cause 50% of cases of non-menstrual TSS. These toxins are super antigens, T cell mitogens that bind directly to invariant regions of major histocompatibility complex class II molecules, causing an expansion of clonal T cells, followed by a massive release of cytokines. This cytokine release mediates the TSS (Salyers and Whitt, 2002; Loir *et al*., 2003).

2.4.4. Epidemiology

Staphylococcus aureus is one of the most important bacterial foodborne pathogen globally. About a quarter of people among the world population carry one or other strain at any one time, and, if they develop an infection, their own colonizing strains are likely to be responsible for such an infection (Kloos and Bannerman, 1994; Quinn *et al*., 1999; Johnson *et al*., 2006).

The clinical significance of *Staphylococcus aureus* is largely due to its ubiquity. It forms parts of the bacterial environment of animals and humans through out the world and can exist as a persistent or a transient member of the normal flora of the skin and mucous membranes without causing any symptoms of diseases (Foster, 1991; Acco *et al*., 2003). In humans, most frequently it is present on the mucus membranes of the nose and throat and in the pores and hair follicles of normal skin, particularly in damp areas such as axillae and perineum. Breaks in skin and mucous membranes allow entrance of these organisms into the body where they may cause disease (Acco *et al*., 2003; Lourdes *et al*., 2004).

In many outbreaks of SFP, a human food handler is implicated who contaminates the food and then, under favorable conditions, staphylococci will multiply and produce enterotoxins (Morandi *et*

al., 2007; Chiang *et al*., 2008). It is estimated that 30-80 per cent of the human population are carriers of *Staphylococcus aureus* and of these 50 per cent carry food poisoning strains. Thus, unhygienic treatment of food has to be considered as a major risk of contamination (Quinn *et al*., 1999; Atanassova *et al*., 2001). Approximately 30 % of the human populations have small number of *Staphylococcus aureus* in the intestine. If the normal flora is disturbed, as can happen after antibiotic therapy, *Staphylococcus aureus* may become a dominant organism for short periods in the intestine and excreted in very large numbers (Jay, 2000; Baron, 2007).

Washing the skin with soap and water usually eliminates many of the gram-negative bacteria but gram-positive cocci tend to rise to the surface of the skin from pores and can be present in even larger numbers on the surface after washing. Scrubbing disturbs the superficial layers of the skin and may further spread *Staphylococcus aureus.* The salt tolerance of the *Staphylococcus aureus* gives them a selective advantage on the skin, as the sweat has a high salt content (Quinn *et al*., 1999)

Most commonly clinical isolates are from the respiratory tract and the skin (pimples, carbuncles, furuncles, suppurative wounds etc.) of humans and animals (Acco *et al*., 2003; Bania *et al*., 2006). Because *Staphylococcus aureus* is major cause of hospital acquired (nosocomial) infection of surgical wounds and community acquired infections, it is necessary to determine the relatedness of isolates collected during the investigation of an outbreak (Freeman, 1985; Foster, 1991).

The sources of infection are mainly contaminated foods, water and environment where the animals are crowded together. The two most important sources to foods and water contamination are nasal carries and individuals whose hands and arms are inflicted with boils and carbuncles and are permitted to handle foods (Hagstad and Hubbert, 1986; Acco *et al*., 2003; Smith, 2007).

Staphylococcus aureus is most often transmitted by direct or indirect contact with a person who has a discharging wound (septic and non-septic lesions), a clinical infection of the respiratory or urinary tract, or one who is colonized with the organism. It can be carried on the hands of healthcare personnel and food preparers. Contaminated surfaces and medical equipment are also possible sources of staphylococci (Foster, 1991; Aycicek *et al*., 2005; Bania *et al*., 2006).

Foods responsible for SFP outbreaks are often those that have been heated to destroy microorganisms, and then require some food handling and storage at room temperature (Chiang *et al*., 2008).

2.4.5. Food products commonly implicated in staphylococcal food poisonings

Many foods will support growth of *Staphylococcus aureus* and toxin production with the exception of those with a lower PH (< 5.0) or a_w below 8.86 (Hocking and Doyle 1997; Ash, 2008). Semi-preserved products (using salt or sugar) may favor the growth of *Staphylococcus aureus*, for unlike many other organisms, it can tolerate these relatively low water activities. In foods where there is no competing spoilage flora, *Staphylococcus aureus* growth will continue unchecked, unless prevented by low storage temperature (Baird and Lee 1995; Loir *et al*., 2003; Pal, 2007).

In any case, the main sources of contamination to food are humans (handlers contaminate food via manual contact or via the respiratory tract by coughing and sneezing), and contamination occurs after heat treatment of the food. Nevertheless, in foods such as raw meat, sausages, raw milk and cheese, contaminations from animal origins are more frequent and due to animal carriage or to infections such as mastitis (Jay, 2000; Silva *et al*., 2000; Baron, 2007).

The most important factors that contributed to SFP outbreaks are inadequate refrigeration of food, preparing food far in advance of planned service, infected personnel, persons practicing poor personal hygiene during food preparation, inadequate cooking or heat processing and holding food in warming devices at bacterial growth temperatures. Thus, unhygienic treatment of food has to be considered as a major risk of contamination, and SFP is often associated with highly manually handled food (Jay, 2000; Atanassova *et al*., 2001; Loir *et al*., 2003).

Foods that are subjected to post processing contamination (contamination of food during handling stage after cooking) by staphylococci represent a significant health hazard because microbes that would normally out compete these organisms would have been eliminated as the staphylococci are poor competitor in the presence of other microorganisms (Miwa *et al*., 2001).

Staphylococcus aureus grow readily in non-acid cooked foods. Many different foods can be a good growth medium for *Staphylococcus aureus*, and have been implicated in SFP, including raw milk, cream, cream-filled pastries, butter, ham, cheeses, sausages, meat pies, salads, cooked meals and sandwich fillings (Hagstad and Hubbert, 1986; Jay, 2000; Loir *et al*., 2003).

Staphylococcus aureus is frequently associated with dairy cows and the dairy environment and is commonly the etiologic agent of mastitis, a problematic disease often found in dairy herds. *Staphylococcus aureus* may be carried by healthy cows and mastitic dairy cows and can easily be shed into the milk during collection. Contamination of such food products by *Staphylococcus aureus* may also occur during the phase of manufacturing and handling of the final products. Once the milk is contaminated, SEs can be produced when the milk is not cooled quickly and/or is not efficiently pasteurized. In France, 25 out of 149 foodborne staphylococcal outbreaks that occurred in 1999 were attributed to the consumption of raw milk cheeses, and 3 out of 13 were also reported in Italy (WHO, 2000; Srinivasan, 2006; Soejima *et al*., 2007).

2.4.6. Clinical significance

Staphylococcus aureus is among the most significant pathogens causing a wide spectrum of diseases in both humans and animals (Johnson *et al*., 2006; Salandra *et al*., 2008).

Disease in food animals

In food producing animal reservoirs, such as ruminants, *Staphylococcus aureus* presents on the skin and mucosae. In animals, *Staphylococcus aureus* can cause pustular inflammation of the skin and other organs, mastitis being the most serious. It is frequently associated to subclinical mastitis becoming responsible of contamination of milk and dairy products and is of great economic importance to the dairy industry worldwide (Jones, 1998; Salandra *et al*., 2008). Its large capsule protects the organism from attack by the cow's immunological defenses (Hein *et al*., 2005). The infection occurs through the teat canal with the organisms derived from contaminated environment especially from the skin of the udder and teat (Anderson and Pritchard, 2008).

Washcloths, teat cup liners and flies mechanically transmit the infection from cow to cow. Cattle are often infected by humans and the infection is carried from one cow to another by the milkers' hands (Freeman, 1985). There are estimates that 80-100% of all herds have at least some staphylococcal mastitis, with 5 to 10% of cows infected (Anderson and Pritchard, 2008). Herds with excellent milking hygiene practices and management have lower levels of staphylococcal intramammary infections as compared to those herds with poor hygiene or management (Kaloreu *et al.*, 2007). The bacterium produces toxins that destroy cell membranes and can directly damage milk-producing tissues (Jones, 1998). Staphylococcal infections also develop in to metritis, enteritis, ear infections and conjunctivitis (Anderson and Pritchard, 2008).

Disease in humans

Staphylococcal infection presents with a wide range of syndromes in human beings affecting many tissues and caused by three mechanisms: local destruction (abscess), blood spread and toxin production (Loir *et al.*, 2003; Soejima *et al.*, 2007). They cause superficial skin lesions like boils(furuncles), pimples, impetigo, carbuncles and localized abscesses in other sites, deep-seated infections such as osteomyelitis and endocarditis and more serious skin infections such as staphylococcal scalded skin syndrome(SSSS) or furunculosis, hospital acquired (nosocomial)infection of surgical wounds and infections associated with indwelling medical devices. Also result in food poisoning by releasing enterotoxins into food, toxic shock syndrome (TSS) by release of super antigens into the blood stream and urinary tract infections (Loir,*et al.*,2003; Shah, 2003; Todar, 2008).

Staphylococcal food poisoning occurs with the ingestion of contaminated food in which the enterotoxigenic strains of *S. aureus* can multiply reaching about 10^5 CFU/g of food; this bacterial load allows the production of an amount 20ng to 1µg of SE sufficient to determine symptoms in human beings (Quinn *et al.*, 1999; Salandra *et al.*, 2008).

The hazard to public health by ingestion of foods contaminated with *S. aureus* is particularly linked to the ability of 50% of these strains to produce thermostable SEs associated with food poisoning (Quinn *et al.*, 1999; Miwa *et al.*, 2001; Kerouanton *et al.*, 2007).

Staphylococcus aureus is extremely prevalent in atopic dermatitis patients, who are less resistant to it than other people are. It often causes complications. The disease most likely found in fertile active places including, the armpits, hair and scalp. The large pimples that appear in those areas may cause the worst of the infection if popped. This can lead to Scalded skin syndrome (Todar, 2008).

All people are believed to be susceptible to this type of bacterial intoxication. However, the onset and severity of the illness is usually dependent on the individual's susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested and the general health of the victim (Jay, 2000; Acco *et al*., 2003; Walderhaug, 2007).

2.4.7. Public health and economic importance

Staphylococcal infections are frequent, but usually contained by immune mechanisms to the site of entry. The highest incidence of disease usually occurs in people with poor personal hygiene, overcrowding and in children. However, anyone can develop a serious staphylococcal infection including fit young people (Hobbs and Gilbert, 1981; Rho and Schaffner, 2007).

In developing countries, the surveillance system of FBD hardly exists and it is therefore, difficult to estimate the real magnitude of the problem (Rowland *et al*., 1994; Hocking and Doyle, 1997). Even in countries where surveillance services are very efficient, the precise incidence of food poisoning is not known, as outbreaks are often not reported to public health authorities. Hence, the incidence of FBD caused by staphylococci is thought to be much higher than reported since many cases remain undeclared (Jay, 2000; Kerouanton *et al*., 2007; Walderhaug, 2007).

Food borne diseases are a serious and growing problem in the world (Baron, 2007). Reports of the yearly incidence of FBD range from 6.5 to 81 million people affected and as many as 9000 deaths each year. The cost to patients, food producers and the national economy estimated to be 7.7 to 8.4 billion USD per year. In USA, only staphylococcal food poisoning costs US$1.5 Billion annually. The vast majority of cases, however, go unreported. Bacterial pathogens caused 66 percent of the

outbreaks, 87 percent of the cases, and 90 percent of the fatalities (Hobbs and Gilbert, 1981; Kloos and Bannerman, 1994).

However, the change in food supply, the identification of new FBD, and the availability of new surveillance data have changed the morbidity and mortality figures (Jay, 2000; Loir *et al*., 2003). A study from the US Centers for Disease Control and Prevention (CDC) reports that FBD cause approximately 76 million illnesses, 325,000 hospitalizations, and 5000 deaths and costs annually 5-6 billion USD in the United States each year (Jay, 2000). Identified pathogens account for an estimated 14 million illnesses, 60,000 hospitalizations, and 1800 deaths. *Salmonella, Listeria,* and SFP organisms are responsible for 1500 deaths. Unidentified pathogens account for the remaining 62 million illnesses, 265,000 hospitalizations, and 3200 deaths. Overall, FBD appear to cause more illnesses but fewer deaths than previously estimated. Among FBD, SFP is of major concern in global public health programmes (Loir *et al*., 2003; Baron, 2007).

Staphylococcal organisms alone have found to cause hospitalization rates as high as 14%. Although not considered especially lethal, death can ensue if large amounts of SE are ingested: fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive persons such as immunocompromised persons, elderly persons and children (Atanassova *et al*., 2001; Aycicek *et al*., 2005; Kerouanton *et al*., 2007).

2.4.8. Diagnosis

History

Symptoms of FBD associated with staphylococci are not suggestive and have little importance to warrant diagnosis (Loir *et al*., 2003; Johnson *et al*., 2006; Baron, 2007). In the diagnosis of SFP detailed history, including the duration of the disease, characteristics and frequency of bowel movements, and associated abdominal and systemic symptoms, may provide a clue to the underlying cause. The presence of a common source, types of specific food, travel history, and use of antibiotics always should be investigated. Besides, gathering and analyzing epidemiologic data are essential (Hobbs and Gilbert, 1981).

Isolation and identification

Incriminated foods should be collected and examined for *Staphylococcus aureus* or the enterotoxins produced. The latter is especially important when foods that have been heated before consumption are implicated in the outbreak. For some outbreaks, food handlers are also tested to ensure whether they are carriers of the strain responsible (Bautista *et al*., 1988; Rho and Schaffner, 2007).

Then the specimen should be sent to the laboratory for definitive identification by using biochemical or enzyme-based tests. A Gram stain is first performed to guide the way, which should show typical gram-positive bacteria, cocci, in clusters. Secondly, culture the organism in mannitol salt agar, which is a selective medium with 7–9% NaCl that allows *S. aureus* to grow producing yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH. Furthermore, for differentiation on the species level, catalase (positive for all *Staphylococcus* species), coagulase (fibrin clot formation, positive for *S. aureus*), DNAse (zone of clearance on nutrient agar), lipase (a yellow color and rancid odor smell), and phosphatase (a pink color) tests are all done(Rho and Schaffner, 2007).
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The presence of relatively large numbers of enterotoxigenic *S. aureus* is good circumstantial evidence that the food contains toxin. The most conclusive test is the linking of an illness with a specific food or in cases where multiple vehicles exist, the detection of the toxin in the food samples (Martın *et al*., 2004; Chiang *et al*., 2008). In cases where the food may have been treated to kill the *S. aureus*, as in pasteurization or heating, direct microscopic observation of the food may be an aid in the diagnosis (Hagstad and Hubbert, 1986; Hein *et al*., 2005).

Rapid Diagnosis

Diagnostic microbiology laboratories and reference laboratories are of important for identifying outbreaks and new strains of *S. aureus*. Recent genetic advances have enabled reliable and rapid techniques for the identification and characterization of clinical isolates of *S. aureus* in real-time. These tools support infection control strategies to limit bacterial spread and ensure the appropriate use of antibiotics. These techniques include Real-time PCR and Quantitative PCR and are

increasingly being employed in clinical laboratories (Omoe *et al.,* 2005; Mackay, 2007; Ash, 2008).

A number of serological methods based on monoclonal antibodies (e.g., ELISA, ELFA, Reverse Passive Latex Agglutination) for determining the enterotoxigenicity of *Staphylococcus aureus* isolated from foods as well as methods for the separation and detection of toxins in foods have been developed and used successfully to aid in the diagnosis of illness. These rapid methods can detect approximately 1.0 nanogram of toxin/g of food (Bania *et al*., 2006; Walderhaug, 2007; Ash, 2008).

2.4.9. Management strategies

Treatment

The objective of treatment in human patients is to replace fluids, salt, and minerals that are lost by vomiting or diarrhea (Foster, 1991; Sandel and McKillip, 2004). Some strains of *Staphylococcus* have acquired genes making them resistant to multiple antimicrobial agents. These organisms are uniformly resistant to penicillins and cephalosporins. Penicillinase-resistant penicillins such as oxacillin and flucloxacillin are used for serious infections. First or second generation cephalosporins such as cephalothin, cephalexin and cefuroxime are usually safe in patients who are hypersensitive to penicillins. Vancomycin is usually effective for methicillin-resistant staphylococci. Erythromycin and its newer relatives are used in milder infections. The infections can also be treated with combination therapy using sulfa drugs and minocycline or rifampin (Kloos and Bannerman, 1994; Rho and Schaffner, 2007).

Prevention and control

Control is both important and difficult as staphylococci can persist for months in dust, curtains and human carriage is often permanent. Reservoirs and routes of spread differ, so different measures are appropriate in different circumstances. Prevention is much concerned with the destruction of the bacteria and with the inhibition of growth (Hocking and Doyle, 1997; Loir *et al*., 2003; Baron, 2007; Chiang *et al*., 2008).

Effective methods for preventing SFP are aimed at eliminating contamination through high standards of personal hygiene to prevent food contamination by food handlers, public education in relation to hand washing, wearing gloves during food preparation and storing foods at proper temperature. Storing foods at temperature less than 4.4 ^{O}C or greater than 60 ^{O}C effectively prevents replication of staphylococcal organisms and significant toxin production (Hocking and Doyle, 1997; Salyers and Whitt, 2002; Ash, 2008).

This inhibits growth or destroys the pathogen and minimize toxin production as heating food after toxin is formed will not be an effective control measure. Moreover, persons with lesions containing purulent exudates should not be permitted to handle food until proper medical advice is sought (Acco *et al*., 2003). In general, it is possible to prevent the contamination of food with *Staphylococcus aureus* before the toxin production by serving hot meal immediately, reheating cooked foods thoroughly, Store cooked food in a wide, shallow container and refrigerate as soon as possible, proper washing of hands and under fingernails before and after food preparation and avoiding food service worker with skin infections in food establishments. Also, keeping kitchens and food-serving areas clean and sanitized and using clean utensils and equipments. If food is to be stored longer than two hours, keeping hot foods hot (over 140°F) and cold foods cold (40°F or under). These all will certainly reduce the incidence of food poisoning outbreaks due to *Staphylococcus* (Jay, 2000; Acco *et al*., 2003; Baron, 2007).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in and around Debre-Zeit town, from October 2009 to March 2010. Debre-Zeit is located at 9ºN and 40ºE, in Oromia National Regional State about 47 km southeast of the capital city of Ethiopia, Addis Ababa. It has a human population of about 95,000. The altitude is about 1850m above sea level. It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September (of which 84% of rain is expected) and a short rainy season from March to May with an average annual rainfall of800mm. The mean annual minimum and maximum temperatures are 12.3^{o} C and 27.7^{o} C, respectively, with an overall average of 18.7ºC. The highest temperatures recorded in May and the mean relative humidity is 61.3%. Debre- Zeit is the center of Ada'a Liben woreda. The Woreda has a total land area of about 1610.56 Km^{2} and divided in to three agro-ecological zones namely midland (94%), highland (3%) and lowland (3%) (CSA, 2006). The study area is shown in figure 1.

DEBRE ZEIT
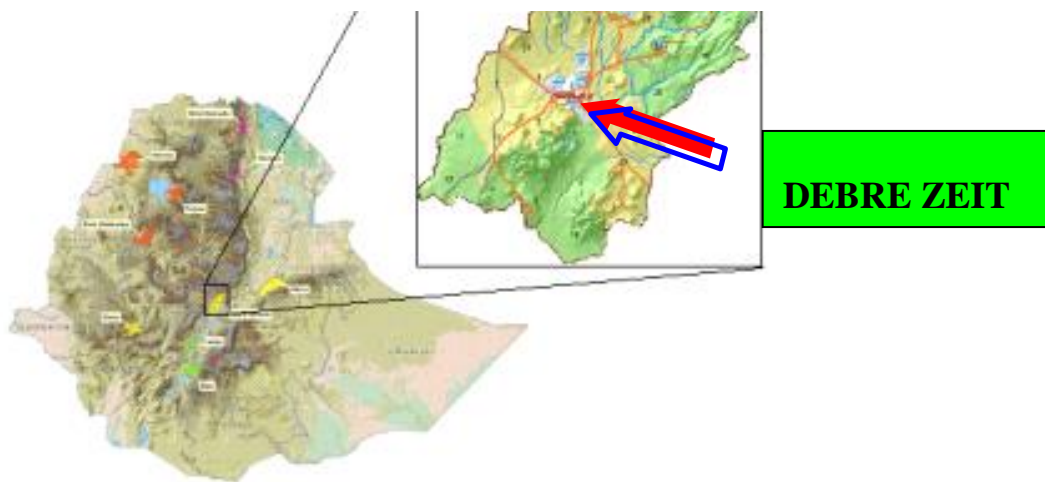
Figure 1: Map of the study area

3.2. Type and origin of samples

The study was conducted on raw bovine bulk milk from the milk cans at farm and tank milk at collection centers and on pasteurized milk. The bulk milk samples were collected from Adaa-Liben district dairy and dairy product producer and marketing co-operative society dairy farms and collection centers and the pasteurized milk was purchased from the collection centers (milk shop) of the cooperative. The cooperative has around three hundred sixty eight members having a dairy farm that were operational during the study period. The dairy farms supply milk to fourteen collection centers.

3.3. Study design

A cross sectional study was conducted from October 2009 to March 2010 to assess the risk of consuming informally marketed raw milk contaminated with *staphylococcus aureus* in Debre-zeit. The study employed a participatory risk assessment approach following the method recommended by the Codex Alimentarius Commission (CAC, 1999; CAC, 2007; FAO, 2006).

3.3.1. Participatory Risk Assessment

Participatory Risk Analysis is a new methodology that combines conventional risk analysis with participatory methods, in order to increase stakeholder engagement in risk analysis while decreasing the need for scarce and expensive resources. Rapid rural appraisal and questionnaire interview (n=218) were undertaken with farmers(170), milk collectors(14), consumers(25), workers in hotels(8) and in the processing plant(1) to generate reliable information on demography, socio-economic, dairy market chains and behavior of handling of milk (hygienic status, transportation, storage temperature and time) and consumption of dairy products. The daily production of milk in and around Debre Zeit was estimated based on available records from Ada dairy cooperative farms, Genesis farms, survey result and expert opinion.

3.3.2. Risk assessment framework

Hazard identification

Staphylococcus aureus was identified as a potential hazard in causing food intoxication particularly with relation to raw milk and milk products based on literatures from scientific findings.

Exposure assessment

Dairy value chain (Figure 2) and a fault tree (Figure 3) were constructed based on the steps involving informal milk marketing pathway in and around Debre-Zeit through RRA   by interviewing key informants (agents along the chain). The fault tree describes the occurrence of hazard and from there describes events that must have occurred for the hazard to be present (Lindqvist *et al*., 2002). The quantities of milk passing through dairy value chains were modeled deterministically. Data collected through interview and laboratory results (prevalence) were used to model the risk of staphylococcal poisoning.

The growth of *S. aureus* and production of entero-toxins were modeled based on the mathematical model and parameters given by Fujikawa (2006).

$dN/dt = rN(1-N/N_{max})\{1-(N_{min}/N)^{c}\}$,

 Where, N is the number of cfu/ml (the population of *S. aureus* at time t), r is the rate constant, or the maximum specific rate of growth, C is an adjustment factor.

The temperature at which milk is exposed to *S. aureus* was modeled as follows. Milking was done twice a day (morning and evening) but the increase of *S. aureus* population can be modeled starting only for one specific time. Therefore, the scenario of morning milking was used to model the risk. The first 3 hours after milking, it is likely that milk is outside a house. It is a warm hours of each day and the temperature was modeled using normal distribution of mean 20°C with standard deviation (SD) 1.5. From the 4th hour, temperature was modeled using normal distribution with the mean 17.1°C and standard deviation 1.7. The mean was brought from

NMSA/ILRI (1998-1999) and the SD was modeled assuming from the figure given in an associated paper (Zippel and Ludders, 2002). The temperature inside refrigerator was modeled as 4°C without uncertainty.

The minimum CFU / ml (Nmin) was modeled according to the formula set by Fujikawa (2006) as indicated below.

$$Nmin = (1 - 1/10^6) \times N0$$

N0, the cfu/ml in a milk container at the time of milking, is modeling only for the contaminated milk with *S. aureus*. According to Middleton (2004), following an artificial intramammary Staphylococcal infection, shed milk contains 10^6 cfu/ml of *S. aureus*. The present study sample one loop (0.01ml) of milk, and therefore 'positive' means picking one or more than one bacteria per 0.01ml, which means more than 10^2 cfu/ml. However, practically one bacteria could be picked up in the lower concentration than that. In the present study, considering these factors, although subjective construction, log of N0 was modeled using normal distribution with mean 3.5 and SD 0.8.

The growth of *S. aureus* was modeled manually by calculating the growth of every hour until 109'th hour, which is the 24 o'clock of the 5th day of milking. The cfu/ml of *S. aureus* at different groups of households as to the maximum storage time (less than 1 day, 1 to 2 days, 3 to 4 days, more than 4 days) and storing in room temperature or refrigerator was modeled using bootstrap of the simulated cfu/ml in two scenarios of storage temperature. There are reports which indicate that *S. aureus* enterotoxins can be produced from 10^5 cfu/ml; however the present study modeled the starting concentration of toxin production as 10^6.5 based on Fujikawa's report (2006) and also to be conservative rather than over-alarming.

Probabilities of boiling and contamination rate of milk with *S. aureus* were used to model the quantity of milk contaminated with *S. aureus* using beta distribution. The quantity of milk contaminated with SEs was modeled using quantity contaminated, probability of belonging in one of the different groups of households as to the maximum storage time and temperature and

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probability that the milk consumed by the group is contaminated with SES simulated by the Fujikawa's model. The probability of belonging one of the different storage time and temperature groups was modeled using Dirichlet Distribution (Vose, 2000) based on data obtained from interviews with farmers. The model was run for 10,000 iterations of Monte Carlo Simulation in @Risk (Palisade) to simulate the quantity and proportion of milk contaminated with *Staphylococcus aureus* entero-toxin (SEs).

$$Qcont=\sum Qi*Ci*(1-Bi)$$

Qcont is the quantity of milk contaminated with *S. aureus*, i is a pathway reaching to consumers.

When: Q*i* is a quantity of milk distributed through a pathway i

C*i* is a contamination rate of milk distributed through a pathway i

B*i* is a probability of boiling milk before consumption at the household level

$$QcontSEs=\sum Qcont \times pGj \times pSEsprodj$$

where: QcontSEs is the quantity of milk contaminated with SEs.

j presents the group of storage time and storage temperature

pGj is the probability of being the group j.

pSEsprod j is the probability that the milk consumed by the group j is contaminated with SEs.

Hazard characterization

The hazard characterization considers characteristics of the host-agent-food matrix; in particular the different types of milk product and implications for survival of *S. aureus.* Information on dose-response was obtained from the literature. The hazard was characterized based on the literature and the present study qualitatively.

Risk characterization

Integrating the steps of hazard identification, hazard characterization and exposure assessment allows us to quantitatively assess the risk. The risk was assessed based on data on the prevalence of *S. aureus* detected in farm bulk milk and collection centers bulk milk, raw milk consumption habit, milk handling practices and quantity of milk at the point of exposures to raw milk. As little amount of SEs can cause poisoning to the consumers, the daily incidence of SEs poisoning was modeled by dividing the quantity of contaminated milk with SEs by the average individual milk consumption deterministically.

3.4. Sampling and sampling size

Stratified random sampling technique was employed to take farm milk samples from milk cans at the farm. The collection centers were taken as strata. To calculate the total sample size, the following parameters were pre-determined: 95% level of confidence (CL), 5% desired level of precision, size of study population 368 (farms currently supplying milk to collection centers of the cooperatives) and with the assumption of 29.1% (Tesfaye, 2008) expected prevalence of *S. aureus* in bulk milk at farm level. Then, the sample size was determined using the formula for sampling from finite population recommended by Thrusfield (2005).

$$n_{adj} = \frac{N \times n}{N + n}$$

Where, n $_{adj}$ is adjusted required sample size in finite population, *n* is the sample size based on an infinite population and *N* is the size of the study population.

Accordingly, the study involved 170 samples, which were proportionally attributed to each of fourteen-collection centers to determine the prevalence of *S. aureus* at farm level and among the collection centers (Table 1).

Table 1. Number of raw bulk milk samples collected from dairy farms

Collection centers	Number of farms	Number of samples
01 kebele 1	40	19
02 kebele	60	28
03 kebele	37	17
08 kebele	12	6
11 kebele	35	16
05 kebele 1	18	8
15 kebele	22	10
06 kebele	38	18
Babogaya*	20	9
01 kebele 2	6	3
05 kebele 2	14	6
Dankaka*	35	16
ILCA*	16	7
Medanialem*	15	7
Total	368	170

Purposive sampling technique was used in sampling raw bovine bulk milk and pasteurized milk from the collection centers (milk shop) to assess the contamination rate of *S. aureus* resulting from cross contamination and post pasteurization contamination, respectively. Accordingly, 25 raw bulk milk samples from collection tanks and 20 pasteurized milk samples were collected from five collection centers (Table 2).

Table 2. Number of milk samples collected from milk collection tanks and pasteurized milk samples at collection centers

Collection centers	Number of tanks(50L)	raw bulk milk	Pasteurized milk
02 kebele	6	6	4
03 kebele	4	4	4
08 kebele	2	2	4
11 kebele	7	7	4
06 kebele	6	6	4
Total	**25**	**25**	**20**

3.4. Sample collection and transportation

Bulk milk samples from the dairy farms (n = 170) and collection centers (n=25) and pasteurized milk from milk shop (n=20) were examined for *S. aureus*. Each sample was collected in a sterile snap-cap milk collection vial from each of the 170 dairy producers and the 5 collection centers during the study period.

Milk samples were collected following the National Mastitis Council (1999) standards for bulk tank milk sample collection and handling. Briefly, milk in the bulk containers were agitated before collection, and samples taken from the top of the bulk tank using a sanitized dipper. Identification of samples were made by date of collection and sources (farm name and Milk collection centers (MCC)) of the milk. All samples were kept in an icebox containing ice packs and taken immediately to the microbiology laboratory of Addis Ababa University, school of Veterinary Medicine, Debre-Zeit for microbiological analysis. Upon arrival, the samples were stored overnight in a refrigerator at $4\,^{o}C$ until examined the next day. Pasteurized milk samples were purchased from the milk shop and immediately transported to laboratory and cultured for bacteriological analysis.

3.5. Isolation and identification of *S. aureus*

3.5.1. Culturing

Isolation and identification of *S. aureus* was conducted in the Microbiology Laboratory of the school of Veterinary Medicine of Addis Ababa University. The bacteriological culture was performed following the standard microbiological technique recommended by Quinn *et al.* (1999). A loopful of milk was streaked on sterile 5% sheep blood agar and the plates were incubated aerobically at 37 ^{O}C and examined after 24-48hrs of incubation for growth. The colonies were provisionally identified based on staining reaction with Gram's stain, morphology and hemolytic pattern. The representative colonies were sub cultured on blood agar plate and nutrient agar plates and incubated at 37 ^{o}C for 24hrs. Pure colonies were preserved and maintained for characterizing the isolates on nutrient slants. Thereafter, the following biochemical tests were done for identification(Annexe 1).

3.5.2. Catalase test

The culture to be tested for catalase test was picked up by bacteriological loop from the agar slant and mixed with a drop of 3% hydrogen peroxide on a clean slide. If the organism is positive, effervescence of oxygen is liberated within a few seconds. Those positive cocci were considered as *Staphylococci* (Quinn *et al.,* 1999).

3.5.3. Mannitol fermentation test

The colonies that were confirmed by staining reaction and catalase test were streaked on mannitol salt agar plate, incubated at 37 ^{o} C, and examined after 24-48 h for growth and fermentation. The presence of growth and change of pH in the media (red to yellow color) regarded as presumptive identification of *S. aureus* or coagulase-positive *Staphylococcus* (Quinn *et al.,* 1999).

3.5.4. Coagulase test

Coagulase test was determined by the method described by Quinn *et al.* (1999). This test was preformed as a tube coagulase test. The selected *Staphylococcus* was subcultured into brain heart

infusion broth and incubated at 37 $^{\circ}$C for 24hrs. Then, 0.5 ml of broth culture and 0.5 ml of sterile rabbit plasma were put into a narrow sterile tube along with a control tube containing a mixture of 0.5 ml of sterile Brain Heart Infusion broth and 0.5 ml of rabbit plasma were incubated at 37 $^{\circ}$C and examined after 4 and 24hrs of incubation and observed for the clot formation. Any coagulation of plasma regarded as positive at either of the readings when compared to the control.

3.5.5. Maltose fermentation test

This test was carried out by using commercially available purple agar base (Difco) with the additional one percent maltose to differentiate the pathogenic S. *aureus*. The suspected culture was inoculated on purple agar base media plate with 1% of maltose and incubated at 37 $^{\circ}$C for 24hrs. Rapid fermentation of maltose by *S. aureus* caused yellow discoloration of the medium due to change in pH (Quinn *et al.,* 1999).

3.6. Data management and analysis

Laboratory analysis results and data collected by interviews were entered into MS-EXCEL and analyzed with statistic SPSS version 15.0. Prevalence of was computed as the number positive samples for *S. aureus* by divided the total number of samples examined in each type of samples items. The Chi-square test was applied to determine existence of any association between risk factors and consumption of raw milk and to see the difference in the contamination rate of *S. aureus* among the collection centers and between urban and peri urban farming. To see the significance of the association between variables Generalized linear model analysis was used. Moreover, to see the effect of some factors on a dependent or response variable that may be influenced by the presence of other factors through effect modifications (i.e. interactions), was further analyzed by multivariable logistic regression. Deterministic model was used to determine the quantity and proportion of milk contaminated with *Staphylococcus aureus* entero-toxin (SEs) and Monte Carlo Simulation was used to simulate it. The daily incidence of SEs poisoning was modeled by dividing the quantity of contaminated milk with SEs by the average individual milk consumption deterministically. $P < 0.05$ was taken as significant.

4. RESULTS

4.1. Rapid rural appraisal and questionnaire interview

4.1.1. Dairy farms and producers

Farming systems

Among the dairy farms (n=170) included in the study, 131(77.1%) were urban dairy farms and 39(22.9%) were peri urban dairy farms. Of the urban dairy farms, 92.4% (121/131) were smallholder dairy farms having crossbreed (Holstein-Friesian x indigenous) and the rest (7.6%, 10/131) kept indigenous breed of lactating dairy cows. On the other hand, all of peri-urban dairy farms (n=39) kept indigenous cattle. The urban dairy farms kept cattle near their house in simple barn shed in zero grazing system. Whereas the peri-urban dairy farms kept in free grazing production systems (extensive production), where animals graze outdoor without feed supplement and confined in simple enclosures near the living compound during night.

Operation of dairy production and sales destinations

All dairy cow owners milk their cows by hand and 70.6% (n=120) use metallic and the rest 29.4% (n=50) use plastic bucket for milking and keep until delivery to the collection centers without cooling. They supply the milk to their association's nearest milk collection center twice a day (morning and evening).

Of the daily milk production, peri-urban farmers used 20% for home consumption, 50% for traditional cheese and butter production and 30% for sales to the collection centers. In urban areas, farmers used 10.5% for home consumption, 63.3% for supply to the collection centers, 10.5% sales to neighbors on contractual basis and 15.7% sales to hotels. The average daily milk consumption by urban dairy farmers was 2 liters where as the average milk sale to hotels and neighbors were 3 and 2 liters per household, respectively.

All the farms included in this study used plastic containers for transporting milk to collection centers. They used clay pot 24.1% (n=41), plastic 19.4% (n=33) and mixing bowl 27.1% (n=46) to store milk at home until consumed and mainly for production of yogurt. The rest 29.4 %(n=50) did not store milk at home. About forty six percent (46.5%) of the farmers (n=79) stored milk at room temperature and while 24.1% (n=41) of them stored milk at refrigeration temperature (+ 4°C). The proportion of farmers who stored milk at room temperature was significantly higher than those who stored at refrigeration temperature (x^2=13.9, df=2, p=0.001). The storage time varies among the farmers and between room temperature and refrigerator. Of the farmers storing milk at room temperature, 24.1% (n=19), 68.4% (n=54) and 7.6%(n=6) stored for one to two days, three to four days and more than four days, respectively. Similarly, of the farmers storing milk at refrigeration temperature, 87.2% (n=34) and 12.8% (n=5) stored for one to two days and three to four days, respectively (Table 4)

Table 3. Proportion of farmers storing milk at various temperatures and time

Storage time(day)	Farmers Storing (%)		Total (%)
	Room temperature	Refrigerator(4°c)	
One-two	19(24.1)	34(82.9)	53(44.1)
Three-four	54(68.4)	5(12.1)	59(49.2)
>four	6(7.6)	2(5.0)	8(6.7)
Total	79(100)	41(100)	120(100)

Consumption of raw milk

Of the 170 dairy producers surveyed, 54 (31.8%) producers reported that they consumed raw milk. They preferred raw milk to boiled milk because of the raw milk attributes such as having good taste, making people healthier and its good nutritional value. All of the farmers interviewed responded that they consume milk products made of raw milk like cheese, yogurt, and butter.

Several factors may be associated with consumption of raw milk. To investigate risk factors for the behavior, firstly each factor was tested in univariate analysis in Generalized linear model and secondly multivariate logistic regression analysis was performed to see interactions and confounding among such factors.

Main source of income

There was no significant difference in the proportion of dairy farmers consuming raw milk according to the main source of income ($P > 0.05$) (Table 5).

Table 4. The proportions of dairy producers consuming raw milk according to the main source of income

Income	Consumption of raw milk			P-value
	Yes (%)	No	Total	
Mixed crop livestock farming	33(94.2)	2	35	0.990
Small trading	3(30.0)	7	10	0.987
Daily labor	2(100)	0	2	0.988
Pension	6(10.9)	49	55	0.986
Government employee	3(18.7)	13	16	0.987
Dairy farming	7(13.5)	45	52	0.986
Total	**54(31.8)**	**116**	**170**	

There was a significant difference in the proportion of dairy producers consuming raw milk among different groups of education level ($p<0.001$). The behavior of raw milk consumption was the most common in illiterate group (62.9%, p=0.014) and the proportion was significantly higher than the other education level groups: elementary (13.2%), secondary (11.9%) and college (23.1%, Table 6).

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Table 5. The education level of dairy producers who consumed raw milk and who did not

Education	Consumption of raw milk			P-value
	Yes	Total	Percentage	
Illiterate	39	62	62.9	0.014*
Secondary	5	42	11.9	0.326
Elementary	7	53	13.2	0.380
College	3	13	23.1	0.067
Total	54	170	31.8	

* Significant

The proportion of dairy farmers consuming raw milk was significantly higher in peri-urban areas (37/39, 94.8%) than urban areas (17/131, 13.0%, x^2=89.25, df=1, p<0.001, Table 7).

Table 6. Two by two table showing the relationship between level of urbanity and consumption of raw milk

Urbanity	Consumption of raw milk		
	Yes	No	Total
Urban	17	114	131
Peri-urban	37	2	39
Total	54	116	170

As the level of urbanity may be influencing the relationship between level of education and proportion of producers consuming raw milk, the relationship between the level of education and level of urbanity was also tested. The proportion of literate people was significantly higher in urban areas 97.2 % (105/108) than in peri-urban areas 2.8 (3/108), x^2=65.01, df=1, p<0.001, Table 8). This factor was taken into account in the multiple regressions below.

Table 7. Two by two table showing the relationship between level of education and level of urbanity where producers reside

Education	Location		
	Urban	Peri-urban	Total
literate	105	3	108
Illiterate	26	36	62
Total	131	39	170

In order to differentiate risk factors of consumption of raw milk from interaction and confounding effects, logistic regression was performed using stepwise model simplification. Tested factors were level of urbanity, main source of income and literacy, and interactions between each combination of two factors and interaction among all three factors. All combinations of interaction were removed at first as there was no significant interaction, and the removal of main source of income (p=0.38) and literacy (p=0.37) did not reduce deviance of the model significantly in Chi-squared test. Finally, the only significant risk factor of consuming raw milk was residing in peri-urban areas ($p<0.001$).

Awareness and knowledge of staphylococcal food poisoning

Only 14.1% (n=24) of the 170 dairy producers surveyed were aware of the occurrence of food borne poisoning due to raw milk consumption and all the 170 producers had no knowledge of staphylococcal food poisoning associated with consumption of raw milk and milk products. Table 8 shows the association between the awareness of food poisoning due to raw milk consumption and behavior of consuming raw milk. There was statistically no difference in the proportions of farmers consuming raw milk between the farmers aware of food poisoning due to raw milk consumption (7/17, 41.2%) and those who are not aware (47/99, 47.5%, x^2=0.003, df=1, p=0.95).

Table 8. Two by two table showing the relationship between awareness of food poisoning among raw milk consuming farmers

Awareness of food poisoning	Consumption of raw milk		
	Yes	No	Total
Yes	7	17	24
No	47	99	146
Total	54	116	170

History of recent mastitis in cows and history of recent food poisoning in humans

Table 10 shows the relationship between history of recent mastitis in cows and history of recent food poisoning in humans at the visited dairy farms. Having mastitis was not statistically associated with the experience of food poisoning (odds ratio= 1.35, 95%CI: 0.644-2.85).

Table 9. Two by two table showing the relationship between history of recent mastitis in cows and history of recent food poisoning in humans

Mastitis	Recent food poisoning in humans		
	Yes	No	Total
Yes	22	130	152
No	2	16	18
Total	24	146	170

4.1.2. Milk Collection centers

During the whole study period, the milk collection centers were observed and the milk collectors were interviewed on the milk handling practices and quality control of milk during collection. The Adaa-Liben District Dairy And Dairy Product Producer And Marketing Co-Operative Society had fourteen collection centers where milk collected from cooperative members twice a day: morning

time from 6:00-7:15 am and evening time from 4:00-5:15 pm. Of the collection centers, four are located near the main roads made of concrete asphalt where as the rest ten are located some distance away the main roads. Four centers were located around the DZ where milk collected from the peri-urban dairy farmers and 10 centers were found in the town where milk collected from urban dairy farmers. Milk collection takes place in simple house made of corrugated sheet.The centers served as both milk collection and milk and milk products sale centers.

All the milk collectors of the cooperative check for the quality of the milk prior to receiving the milk using lactometer reading, Alcohol test and fabric made of cotton to test adulteration with water, freshness and presence of contaminant materials (feces, hay, hair etc) in the milk, respectively. Metallic cans are used for collection of milk at all centers. The cans are cleaned with soap and pipe water at the processing plant and dispatched to the collection centers by vehicles used for transportation of milk to the plant. The cooperative purchased milk only from the farm members with Ethiopian birr (ETB) 5.50 per liter of milk and the price decreases slightly during the fasting period.

Raw milk, pasteurized milk, cheese and butter are sold at these centers to the local community and to hotels, cafeteria and restaurants at sale price of ETB 6.50 and 8.00 per liter and 8.00 per kg, respectively. Of the daily milk collection, 7.3% is sold to the local community, 1.3% to hotels, cafeteria and restaurants and the rest 91.4% sent to the processing plant of the cooperative. Raw milk is kept at room temperature and pasteurized milk and other milk products are kept at 4 ^{o}C in refrigerator until sold

4.1.3. Processing plant

The processing plant of the Adaa-Liben District Dairy and Dairy Product Producers and Marketing Co-operative Society is a modern processing plant located in DZ. It is equipped with facilities like cooling tanks to keep the keeping quality of the milk until processed. The bulk tank milk at collection centers were tested prior reception and transportation to the plant with lactometer reading and alcohol test. Of the milk collected and supplied to the plant, only 39% are processed to produce pasteurized milk, cheese and butter per day. Small quantity of milk (1%) is sold to hotels/cafeteria/restaurants in DZ and the rest 60% is sent to Shola (Mama) Dairy Processing Plant

located in Addis Ababa. The plant failed to process milk to its full capacity due to market insufficiency and lack of sufficient packaging materials. The cooperative sold pasteurized milk, cheese and butter at the processing plant and collection centers to supermarkets and individual consumers.

4.1.4. Consumer

The average quantity of milk bought by the consumers was 2.0 liters per day. Among the consumers, 64% (n=16) used plastic containers and 36% (n=9) glass containers to transport milk to their homes. The time of milk purchase varied among the consumers and 40% (n=10) buy early in the mornings, 16% (4) evenings and 44 %(n= 11) mornings and evenings. Of the consumers, 64 %(16/25) consume boiled milk and the other 36 % (9/25) consume in the form of raw milk and raw milk products like yogurt. The proportions of people who consume raw milk were not significantly different between these consumers (36%) and dairy farmers described above (31.8%, 54/170, x^2=0.038, df=1, p=0.846).

4.1.5. Hotels, cafeteria and restaurants

Except one hotel that buys raw milk from the processing plant (50L per day), all others buy raw milk from the collection centers (averagely 75L per day).They all use plastic containers to transport the milk. Except the hotel that buys milk from the processing plant, which use self-owned vehicle, others use bicycle to transport the milk from the sale points. Milk was bought by all these people early in the morning and sold during the daytime to clients in the form of "Macchiato" (boiled milk+ coffee) and boiled milk alone. Apart from raw milk, the hotels bought cheese and butter from the cooperative. Boiling milk was the common practice in all establishments.

All the respondents know about food poisoning. However, they have no information or awareness about staphylococcal poisoning in relation to unhygienic handling of milk and milk products. Only 25% (n=2) of mangers responded the presence of trained waiters/workers about handling of foods and hotel managements. There was no trend of visit of physicians for regular medical check up of the workers' health status to limit risk of food contamination in all of the establishments.

4.2. Isolation of *S. aureus*

4.2.1. Isolation of *Staphylococcus aureus* from farm bulk milk

Out of 170 raw milk samples tested, 43.5% (74/170) were contaminated with *Staphylococcus aureus*. The frequency of isolation of *Staphylococcus aureus* varied among collection centers. Comparison of the proportion of milk contaminated among different collection centers showed differences in the contamination rate of milk with *S. aureus* (X^2=34.9, df=13, *p*=0.001). Among all the collection centers, the percentage of contaminated milk with *S. aureus* was significantly higher than average at 05 Kebele1 (75.0%, p=0.0498), 05 Kebele 2 (83.3%, p=0.0474), ILCA (85.7%, p=0.0308). A hundred percent of contamination rate was observed at Madenialem milk collection centers (100%), Table 10).

Table 10. Prevalence of S. *aureus* in farm bulk milk among the collection centers

Collection centers	Positive	Total	Percent	*p*-value
01 Kebele 1	6	19	31.58	0.1172
01 Kebele 2	1	3	33.33	0.9517
02 Kebele	8	28	28.57	0.8250
03 Kebele	6	17	35.29	0.8135
05 Kebele 1	6	8	75.0	**0.0498**
05 Kebele 2	5	6	83.33	**0.0474**
06 Kebele	4	18	22.22	0.5235
08 Kebele	4	6	66.67	0.1413
11 Kebele	6	16	37.5	0.7134
15 Kebele	3	10	30.0	0.9304
Babogaya	4	9	44.44	0.5090
Dankaka	8	16	50.0	0.2711
ILCA	6	7	85.71	**0.0308**
Madenialem	7	7	100	
Total	74	170		

The milk produced and collected in peri-urban areas was significantly more contaminated with *S. aureus* (25/39, 64.1%) than milk produced and collected in urban areas (50/131, 38.2%, x^2=7.18, df=1, *p*=0.007). Urban was a preventive factor for milk contamination with *S. aureus* (odds ratio 0.346, 95%CI (0.332, 0.360)) whereas farming in peri-urban areas was a risk factor for contamination of milk with *S. aureus* (odds ratio=2.89, 95%CI: 2.78-3.01).

Table 11. Prevalence of *Staphylococcus aureus* in urban and peri-urban dairy farms

Dairy farm	Positive	Prevalence (%)	Total
Peri-urban	25	64.1	39
Urban	50	38.2	131
Total	75	44.1	170

4.2.2. *Staphylococcus aureus* in milk collection centers bulk milk

Out of 10 urban collection centers, 5 centers (02 Kebele, 03 Kebele, 06 Kebele, 08 Kebele and 11 Kebele) sold raw milk to consumers and the milk sold in these centers were tested for *S. aureus*. Out of 25 raw milk samples tested, 72% (18/25) were contaminated with *Staphylococcus aureus*. The frequency of isolation of *Staphylococcus aureus* varied between 66.7% and 100% among collection centers. However, there was no significant difference in the prevalence among these collection centers (X^2= 1.497, df=4, p=0.827)(Table 12).

Table 12. Prevalence of *S. aureus* in bulk tank raw milk at milk collection centers selling raw
milk directly to consumers

Collection centers	Positive	Total	Percentage
02 kebele	4	6	66.7
03 kebele	3	4	75.0
06 kebele	4	6	66.7
08 kebele	2	2	100
11 kebele	5	7	71.4
Total	18	25	72.0

The farm results from these five collection centers (Table 11) were added up and compared with
the results of samples collected at the collection centers. Accordingly, the prevalence of *S. aureus*
at collection centers (72.0%, 18/25, Table 13) was significantly higher than that at the farm level
(32.9%, 28/85, Table 11, $x^2=10.56$, df=1, $p=0.001$).

Relatively higher *S. aureus* was isolated from dairy producers who consumed raw milk (55.6%,
30/54) than who did not (38.8%, 45/146, Table 14). However, there was no significant difference
in the incidence of *S. aureus* between dairy farmers who did and did not consume raw milk (χ^2
$=3.547$, df = 1, $p=0.60$).

Table 13. Proportion of *S. aureus* between dairy farmers who did and did not consume
raw milk

Consumption of	*S. aureus* (%)		
raw milk	Positive	Negative	Total
Yes	30(55.6)	24(44.4)	54
No	45(38.8)	71(61.2)	116
Total	75	95	170

4.2.3. Isolation of *Staphylococcus aureus* from pasteurized milk

Twenty pasteurized milk samples were sampled from five collection centers to assess the survival of *S. aureus* as result of tolerance of pasteurization temperature and possibility of post processing contamination. *Staphylococcus aureus* was isolated from none of the samples.

4.3. Risk assessment result

4.3.1. Hazard identification

One organism of particular interest to milk food safety is *Staphylococcus aureus*. This facultative anaerobic gram-positive bacterium is a major cause of food borne intoxications and outbreaks throughout the world because of its ubiquity and its ability to persist and grow under various conditions (Lindqvist *et al*., 2002; Kerouanton *et al*., 2007). Many foods will support growth of *Staphylococcus aureus* and toxin production with the exception of those with a lower PH (< 5.0) or a_w below 8.86 (Hocking and Doyle 1997; Ash, 2008). Consumption of raw milk and milk products made of raw milk are associated with a greater risk of SE intoxication and hence the presence of *S. aureus* in raw milk and milk product is a hazard (Rho and Schaffner, 2007).

Staphylococcal food poisoning is characterized by emesis, nausea, vomiting, diarrhea, sweating, abdominal cramping, and prostration in human beings (Jay, 2000; Acco *et al*., 2003). The duration of illness typically is 1 to 2 days. However, it usually takes three days to recovery completely and sometimes longer in severe cases (Jay, 2000; Lindqvist *et al*., 2002; Aycicek *et al*., 2005).

Staphylococcus aureus has found to cause hospitalization rates as high as 14%. Although not considered especially lethal, death can ensue if large amounts of SEs are ingested: fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive persons such as immunocompromised persons, elderly persons and children (Atanassova *et al*., 2001; Aycicek *et al*., 2005; Kerouanton *et al*., 2007).

4.3.2. Hazard characterization

Adverse health effects

The adverse effects from *S. aureus* depend on the virulence (Enterotoxin) of the pathogen, the susceptibility of the host and the dose ingested. *Staphylococcus aureus* can cause SFP by preformed toxin production with an incubation period of 1-6 hours as well as by infecting both local tissues and the systemic circulation with variable and indefinite incubation period, most commonly 4-10 days. Patients become symptomatic after ingestion of thermo-stable SEs of an approximate dose of 0.1 to 1.0mg/kg of body weight and SFP caused by ingestion of this SEs have a rapid onset and include nausea, vomiting, abdominal pain and diarrhea (Miwa *et al*., 2001; Chiang *et al*., 2008).

Unlike other common food borne illnesses that require consumption of and infection by viable pathogenic microbial cells, sickness associated with *S. aureus* occurs following ingestion of numerous heat- and protease-stable staphylococcal enterotoxins (SEs). These produced under specific environmental conditions when the population density of the pathogen reaches 10^5 CFU/ml (Lindqvist *et al*., 2002; Kerouanton *et al*., 2007; Heidinger *et al*., 2009).

When SEs are expressed systematically, they mediate two illnesses, TSS and SSSS. In both diseases, exotoxins are produced during an infection, diffuse from the site of infection, and are carried by the blood (toxemia) to other sites of the body, causing symptoms to develop at sites distant from the infection. Toxic shock syndrome toxin is produced when SEs are expressed systemically and it causes TSS. It is very weakly related to enterotoxins and does not have emetic activity (Bania, 2006; Smith, 2007). Toxic shock syndrome is an acute life-threatening illness mediated by staphylococcal superantigen exotoxins and can occur as a sequel to any staphylococcal infection if an enterotoxin or TSST is released systemically and the host lacks appropriate neutralizing antibodies (Foster, 1991; Salyers and Whitt, 2002).

The existence of long-term effects following *S. aureus* poisoning was not reported. In animal studies, a certain degree of immunity has been shown after repeated exposure to the same type of SEs (Lindqvist *et al*., 2002; Rho and Schaffner, 2007).

Dose response relationship

The relationships between dose and response following consumption of each individual SE were not found in the literature. However, it is estimated that 20ng to 1μg of SE's, particularly SEA, and the pathogen with more than 10^5 CFU/ml can potentially cause illness. Nevertheless, there are both reports of lower and substantially higher levels of SEs required causing illness (Lindqvist *et al*., 2002; Kerouanton *et al*., 2007; Heidinger *et al*., 2009). Considering the mode of vending at collection centers at room temperature, the growth model showed that raw milk sold in informal market of Debre-Zeit can contain *S. aureus* with more than 10^5 CFU/ml.

In the present study, the virulence of *S. aureus and* susceptibility of people were not investigated (i.e. dose-relationship was not established), but considering the adverse health effects, dose-response relationship described above and growth model hazard of *S. aureus* poisoning following consumption of contaminated milk is qualitatively estimated as harmful.

4.3.3. Exposure assessment

The path way or dairy value chain (Figure 1) and a fault tree model (Figure 2) showing source exposure to *S. aureus* were developed based on the steps involving informal milk marketing pathway in and around Debre-Zeit. Along the dairy value chain, it was identified that people exposed to raw milk at farm, collection centers and processing plant. The contamination rate of milk in farm bulk milk and collection centers bulk milk was 43.5% and 72% respectively. However, interview with dairy farmers and consumers showed that 31.8 % of the farmers and 36% of consumers had the habit of consuming raw milk they showing that are more likely exposed to SFP. Among 23,810 L of daily milk production (figure 2), 964.2L (90%CI: 544.1-856.5) contaminated with *S. aureus* and 166.7 L (90%CI: 125.2-213.6), which is 0.7% (90% CI: 0.5-0.8) of the total production, is contaminated with SEs at the time of consumption by consumers.

Figure 2. Dairy value chain and estimated milk quantity per day at each stage of dairy value chain in and around Debre-Zeit

```
                    ┌──────────────────────────────────────────────┐
                    │ Dairy production in and around Debre-Zeit      │
                    │ (23810.6=100%)                                 │
                    └──────────────────────────────────────────────┘
                         │                                    │
           ┌─────────────────────────────┐      ┌─────────────────────────────┐
           │ Urban milk production        │      │ Peri-urban milk production   │
           │ 19257.6L (80.9)              │      │ 4553L (19.1%)                │
           └─────────────────────────────┘      └─────────────────────────────┘
```

| SH 2940 (12.3%) | HC: 1960 (8.2%) | SN1960 (8.2%) | 12397.6 (52.1%) | 1365.9 (5.7%) | HC 910.6 (3.8%) | TP 2276.5 (9.6%) |

```
                    ┌──────────────────────────────────────────────┐
                    │ Sale milk to collection centers                │
                    │ 13,763.5L (57.8%)                              │
                    └──────────────────────────────────────────────┘
```

| Ada milk CC 5,503.5 L (23.1%) | Other milk CC 8,260L (34.7%) |

| SH 75L (0.3%) | SI 400L (1.7%) | PP 5028.5L (21.1%) | Milk processors in Addis Ababa |

| SH 50L 0.2% | Process milk 1978.5L (8.3%) | Supply to Shola dairy industry in Addis Ababa 3000L (12.6%) |

Key: SH= sale to hotels, cafeterias, restaurants etc
SI= sale to individuals who consume at their home
HC= home consumption by the producers
SN= sale to neighbors on contractual basis
TP= milk processed traditionally by rural farmers and sold in the open market in DZ
CC=collection centers
PP= processing plant of Ada dairy cooperative

Figure 3. Fault tree showing the events leading to *S. aureus* poisoning from informally marketed milk in Debre-Zeit
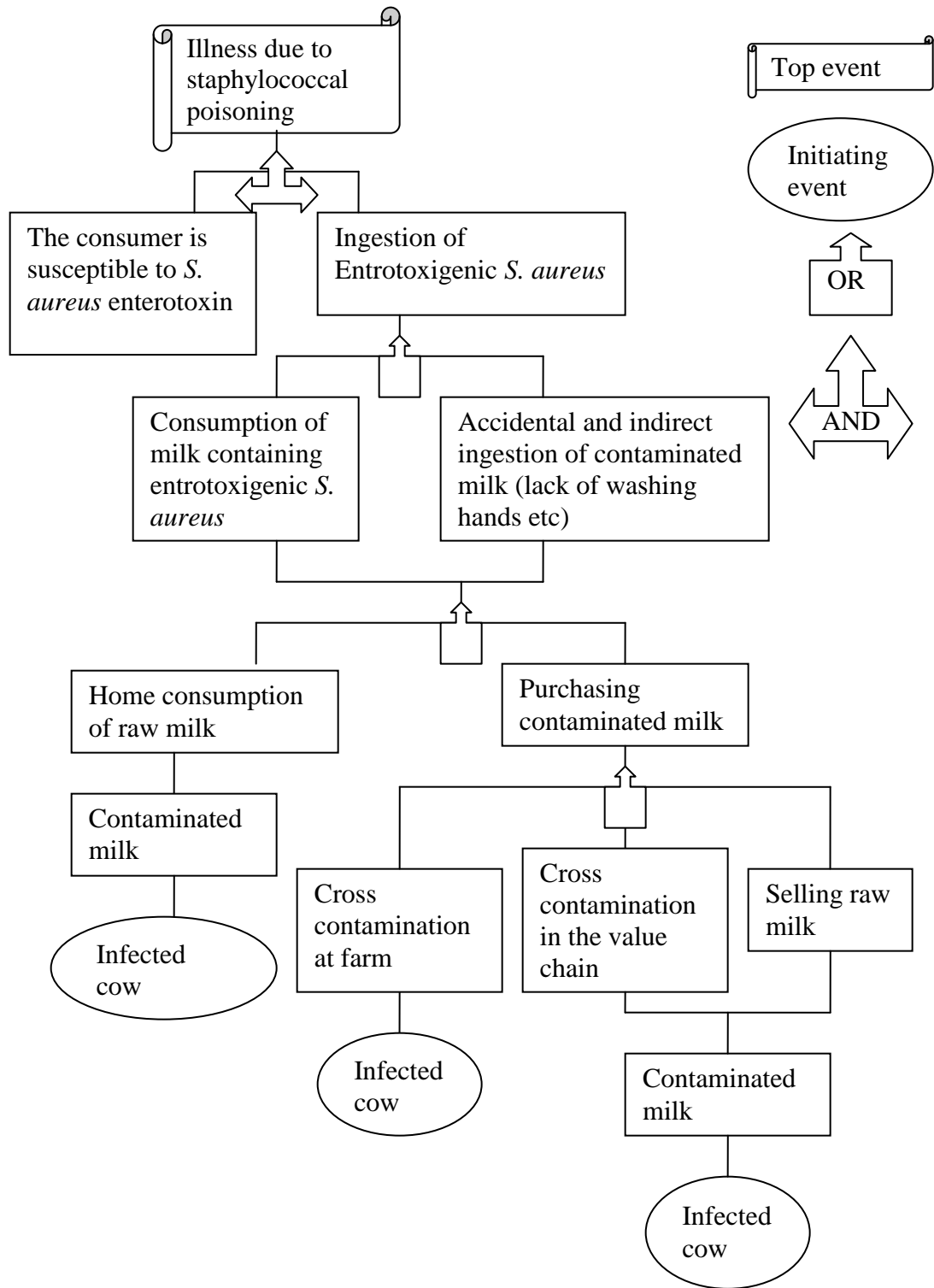
4.3.4. Risk characterization

The risk was assessed based hazard identification, hazard characterization and exposure assessment result. The risk model was constructed following point of exposures (figure 4). The incidence depends on how many people in DZ consume milk. Producers and Consumers consume 2 liters per household per day (survey result). Therefore, if we assume 2 liters per household, and four people per household, then each individual can consume about 0.5 liter per day. As little amount of SEs can cause poisoning to the consumers, everyday 333 (90% CI: 250-427) people could acquire Staphylococcal poisoning in the urban areas of Debre-Zeit (Table 14).

Figure 4. Risk model showing source of exposure to Debre-Zeit population
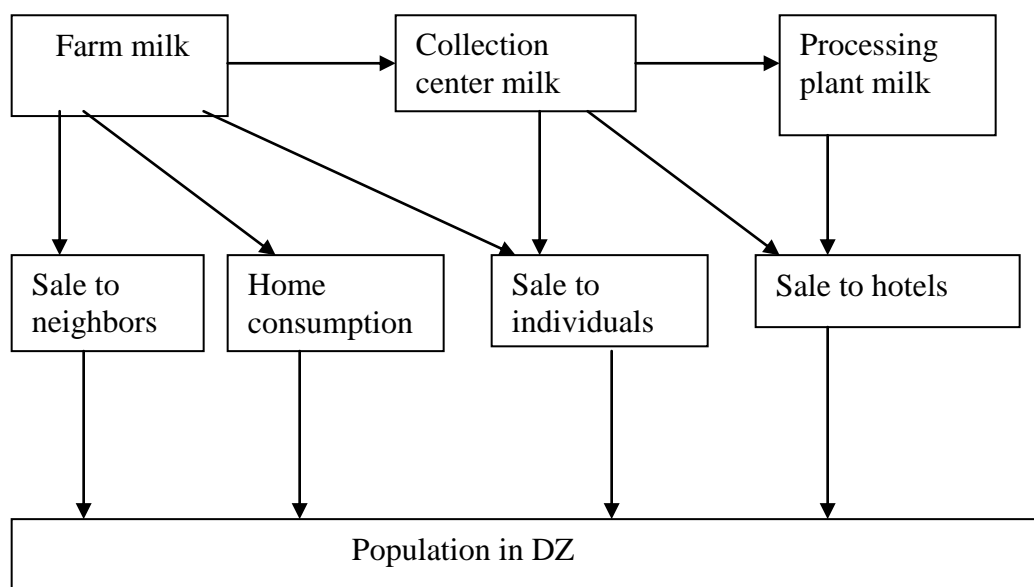
Table 14. **Quantity of milk contaminated with SEs and daily incidence of** *S. aureus* **poisoning**

Description	90% CI		
	Mean	**Lower**	**Upper**
Quantity of contaminated milk with *S. aureus*	694.2	544.1	856.5
Quantity of contaminated milk with SEs	166.7	125.2	213.6
Proportion of milk with SEs	0.7	0.5	0.8
Daily incidence of *S. aureus* poisoning	333	250	427

5. DISCUSSION

Despite the known association of raw milk with pathogenic organisms, some consumers believe raw milk is of better quality than pasteurized milk (Jayarao *et al*., 2006). Dairy producers practiced consumption of raw milk for several reasons. Many farm families consume raw milk simply because it endowed with good flavor, posses natural constituents, offer great satisfaction and is a traditional practice than pasteurized milk. In this study, 31.8 % of dairy producers and 36% consumers surveyed reported that they had the habit of drinking raw milk. A study in the USA also reported that 42.3% of dairy producers consumed raw milk (Jayarao *et al*., 2006). Though the result showed relatively a lower percentage of raw milk consumers, still these individuals are at a greater risk of contracting staphylococcal intoxication than those who do not consume raw milk.

In our study, of factors (income, education, urbanity, and knowledge of food poisoning) supposed to be associated with raw milk consumption, only peri-urban farming was significantly associated with raw milk consumption. This indicated that peri urban farmers are at greater risk of acquiring raw milk related diseases in general and staphylococcal food poisoning in particular.

In this study, All dairy cow owners milk their cows by hand and 70.6% (n=120) use metallic and the rest 29.4% (n=50) use plastic bucket for milking and keep until delivery to the collection centers without cooling. The usage of plastic materials was common during milking, transporting and storing at all stage of dairy value chain. These containers were usually not disinfected. This may lead to easy adherence and multiplication of the microorganisms to the milk containing containers, which in turn ultimately increase the risk of staphylococcal infection and poisoning.

Nearly half of the dairy farmers (46.5%) store milk at room temperature temporarily and to make yogurt. This would certainly support the growth and multiplication of *S. aureus* as it is able to survive and multiply in a variety of food substrates, at a range of 7 to 48 ^{o} C temperatures). The overall effect of these poor milk-handling practices could lead to contamination of the dairy product as investigated in the study.

In this study, *S. aureus* was isolated in 43.5% (74/170) of the farm bulk milk samples and 72 % (18/25) of the milk collection centers bulk milk samples. It was isolated from none of the samples of pasteurized milk. The result showed a high prevalence rate at milk collection centers, which might attributed to cross contamination of milk while bulking and poor handling across the dairy value chain. The prevalence of *S. aureus* at collection centers was nearly in agreement with the pervious work (Wubete, 2004) where *S. aureus* was isolated at recovery rate of 75%. The result of the present study showed a slight lower prevalence rate (43.5%) than a recent report from Norway where *S. aureus* was recovered in 75% of 220 bovine farm bulk milk samples (Jorgensen *et al*., 2005). However, the result was relatively higher than the previous works, 29.1% (Tesfaye, 2008) and 27% (Wubete, 2004) done in the same study area. Pasteurization of commercially distributed milk has greatly reduced the risk of infection resulting from the consumption of contaminated milk (Jayarao *et al*., 2006). The present study found absence *S. aureus* from pasteurized milk samples. This shows that *S. aureus* were inactivated during the pasteurization process and absence of post pasteurization contamination.

The study showed that the proportion of milk contaminated with SEs at the time of consumption was 0.7% (90% CI: 0.5-0.8). Everyday, 333 (250-427) people are suffering from Staphylococcal poisoning in the urban areas of Debre-Zeit. From this, we can extrapolate that the probability of developing the illness among highly susceptible individuals might be much more than this figure. This really indicated that people in the urban are a greater risk of the disease as long as they keep on consuming raw milk.

Only data on the prevalence of *S. aureus,* proportion of milk contaminated, raw milk consumption habits and handling practices were measured. The level or concentration of bacterium and amount of toxin per ml of milk at point of exposure and consumption were estimated using mathematical model. These represent substantial knowledge gap and the way forward for further research. Despite these limitations this type of quantitative assessment was still worthwhile since it was possible to gain insights and to further evaluate several factors that influence the potential risk, e.g. raw milk consumption, contamination rate, milk handling practices and quantity of milk along the dairy value chain in the study area.

6. CONCLUSION AND RECOMMENDATIONS

Milk intended for human consumption must be free from potentially harmful pathogens. Safe and quality milk can only be obtained if effective hygienic control measures are taken throughout the milk chain starting from milking until the milk reaches the consumers mouth. *Staphylococcus aureus* is one of the most important causes of milk borne illness associated with the consumption of raw milk. Quantitative risk assessment is the best tool to estimate the extent of milk borne infections and intoxications. The study undertaken has shown that raw milk produced and sold in informal market in Debre-Zeit area was contaminated with *Staphylococcus aureu*s throughout the milk chain starting at the farm, milk collection centers and dairy processing plants. Consumers in peri urban areas are more likely to acquire the infection than consumers in urban areas. The prevalence of *Staphylococcus aureus* is higher in collection centers than at farms. Despite the limitations and the data gap, we demonstrated the benefit of participatory risk assessment not only as a risk evaluation tool but also as a helping device in the decision-making and the risk management

In light of the present study, the following risk management options were recommended,

- ❖ Raw milk intended for consumption should be subjected to pasteurization or heat treatment at least equivalent to pasteurization temperature
- ❖ Milk should be stored in refrigerator at required temperature until consumption
- ❖ Proper handling of milk along the dairy value chain ought to be exercised to increase the shelf life milk and make it safe for human consumption
- ❖ A well approved quality control measures should be implemented along the dairy value chain particularly at milk collection centers
- ❖ The public health inspectors should examine properly the conditions during production, storage and commercialization of all products made with unpasteurized milk.
- ❖ Considerable research effort is still required for better understanding of the interactions between *Staphylococcus aureus* and dairy products, and of the mechanisms of SEs production in foodstuffs.

❖ Staphylococcal poisoning in relation to milk and milk products should be further conducted taking in to account all possible parameters like the concentration of bacterium, the amount and responsible types of toxins and dose response relationship

❖ Awareness should be made to all stakeholders with regard to milk related SFP so as to manage the risk

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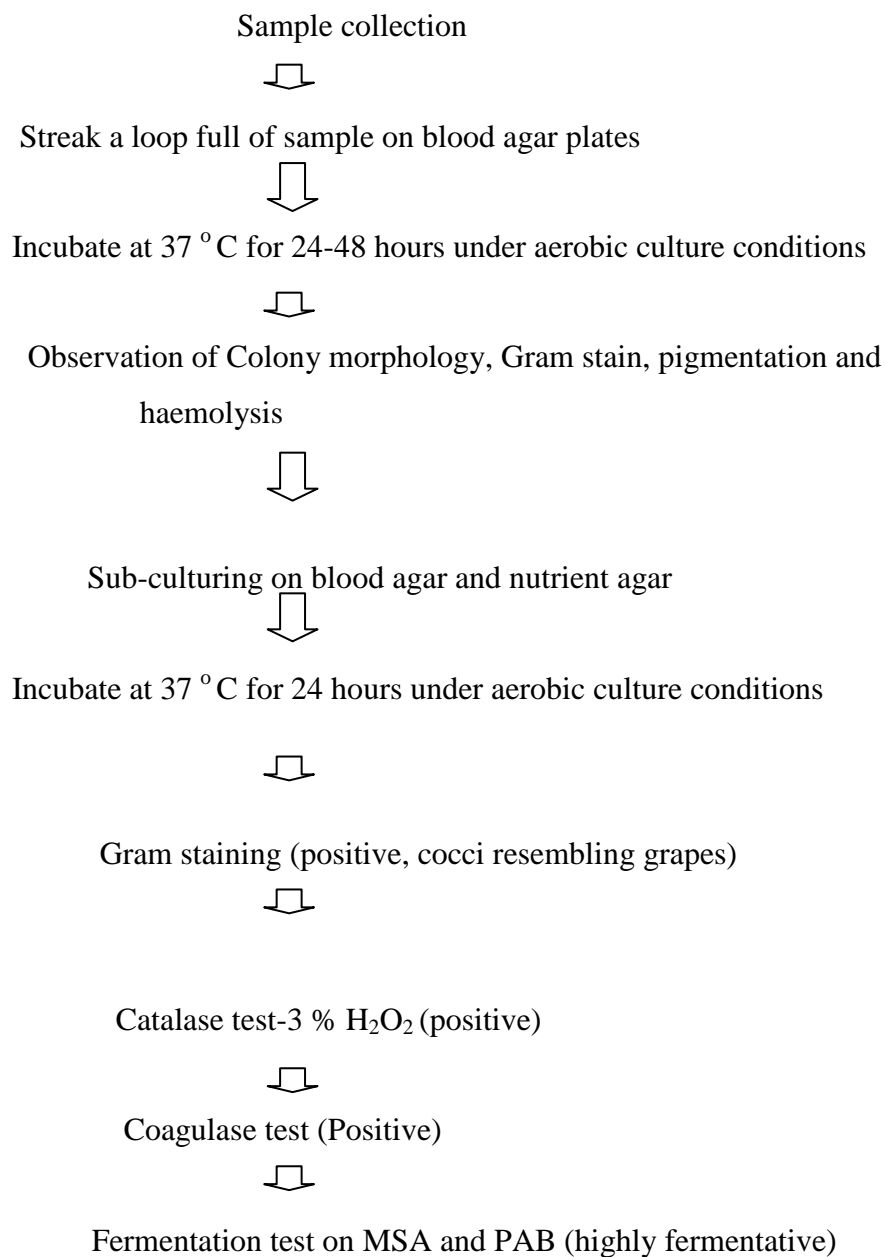
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8. ANNEXES

Annex 1: Flow chart showing procedure for isolation and identification of *S. aureus* from raw bulk milk and pasteurized milk

<div align="center">

Sample collection

⇩

Streak a loop full of sample on blood agar plates

⇩

Incubate at 37 ^{o}C for 24-48 hours under aerobic culture conditions

⇩

Observation of Colony morphology, Gram stain, pigmentation and haemolysis

⇩

Sub-culturing on blood agar and nutrient agar

⇩

Incubate at 37 ^{o}C for 24 hours under aerobic culture conditions

⇩

Gram staining (positive, cocci resembling grapes)

⇩

Catalase test-3 % H_2O_2 (positive)

⇩

Coagulase test (Positive)

⇩

Fermentation test on MSA and PAB (highly fermentative)

</div>

Annex 2: Sample collection sheet for laboratory analysis

SN	Type of sample	Date of collection	Collection center	Sample code	Number of samples

Annex 3: Record sheet for laboratory isolation and identification of *Staphylococcus* aureus

SN	Type of sample	Sample code	Colony characteristics on BAP	Haemolysis	Gram stain	Catalase test	Coagulase reaction	Growth and Mannitol fermentation (MSA)	Maltose fermentation (PAB)	*Staphylococcus aureus*

Annex 4: Procedure for catalase test

1. Place a drop of 3% H_2O_2 on a glass slide.
2. Touch a sterile loop to a culture of the organism to be tested and pick up a visible mass of cells (colony).
3. Mix the organism in the drop of hydrogen peroxide.
4. Observe for immediate and vigorous bubbling.

Interpretation: Bubbling indicates a positive (+) test and no bubbling indicates a Negative (-) test.

Annex 5: Procedures for coagulase test

1. using a sterile pipette, add 0.5ml of the re hydrated plasma to a 12x75mm test tube.
2. Using a sterile serological pipette, add 0.5ml of the overnight broth culture of the test organism to the tube of plasma or, using a sterile bacteriological loop, thoroughly emulsify 2-4 colonies (one loop full) from a non inhibitory agar plate in the tube of plasma.
3. Mix gently and Incubate at $37^{o}C$
4. Examine periodically for coagulation by gently tipping the tube after the first hour and once every hour thereafter until four hours have elapsed. If no clot observed at the end of this period, examine at 24 hours. Avoid shaking or agitating the tube during reading. Doubtful or false-negative results may occur due to breakdown of the clot.
5. Record results: Positive-any degree of clotting - from a loose clot suspended in plasma to a solid clot that is immovable when the tube inverted, Negative - no degree of clotting

Annex 6: Differential tests used for identification of *Staphylococcus* aureus from other staphylococcus species

SN	*Staphylococcus* species	Haemolysis	Pigment production	Coagulase test	Fermentation of sugar MSA	PAB
1	*S. aureus*	+	+	+	+	+
2	*S. intermidius*	+	-	+	$\pm$	$\pm$
3	*S. hicus*	-	-	+	-	-
4	CNS	-	-	-	-	-

+ = 90% or more strains are positive, $\pm$ = 90% or more strains are weakly positive, - =90% or more strains are negative.

Source: Quinn, *et al.*, 1999

Annex 7: Questionnaire format used to interview key informants along the milk pathway

The Milk collection centers Questionnaire

Date: _____ Name: _____

Time: _____ physical address: _____

Collection centers:_____ Sample number: _____

Question	Answer(s)	Remarks
Where do you collect the milk? and How many liters do you collect a day?		
How many days do you work/collect/week?		
Do you test milk when you buy/collect? What kind of test?		

What kind of containers do you use to collect/store milk?		
How long the milk stay at centre? At what temperature?		
Time of collection (morning and evening)?		
What is the price of (raw milk and pasteurized milk)?		
How many liters of raw milk do you sell to individuals and others (e.g. hotels a day?		
Do you sell other dairy products? (Source and quantity)		
Do you consume raw milk? If yes, the rationale?		

The milk producers Questionnaire

   Date:_____Sample number: _____

  Physical address: _____

1. Owner name _____

2. What is your major occupation (means of income)? (1 = dairy farming, 2 = other)

3. What is your educational status? (1 = illiterate, 2 = elementary 3 = secondary 4 = high school, 5 = College)

Question	Answer	Remarks

Number of cattle
  (Cows, calves, bulls and
  heifers)
      and
Cattle breeds		

Quantity of milk (L/day)
    Dry season
    Rainy season		

Destination of sales and means (truck, bicycle, direct)		
How many milk do you deliver to hotels, cc, neighbors and use at home?		
Do you drink raw milk? If yes, what is the reason?		
For how long do you keep milk at home? What temperature?		
Price of milk		
What materials do you use to milk, store and transport?		
History of mastitis		
History food poisoning? If yes symptoms		

The milk consumers Questionnaire

Date: _____Name:_____

Physical address: _____Sample number: _____

Question	Answer(s)	Remarks
Where do you buy the milk? And How many liters do you buy a day?		
What kind of containers do you use? When do you buy?		
How long the milk stay at home prior consumption? At what temperature?		
In what form do you consume (raw, boiled, others)? What is the rationale?		
Do you know any food poisoning/GITdisturbance associated with drinking of raw milk? What are the symptoms?		

The milk processor Questionnaire (milk handling at processing plant)

Date: _____Sample number: _____

Physical address: _____

Question	Answer(s)	Remarks
How milk transported from collection centers? Temperature? Containers?		
How many liters do you collect from the centers? Quantity processed?		
Arrival time of milk(morning, evening)		
How long the milk stay prior processing? Storage tank and temperature?		
What kind of tests used up on arrival?		
What kind of packaging used for pasteurized milk?		
What kind of safety measures used in general?		

Questionnaire for managers/coordinators of hotels, cafeteria and restaurants

Date: _____Sample number: _____

Name: _____ Physical address: _____

Question	Answer(s)	Remarks
Where do you buy the milk? How many liters do you buy a day?		
What kind of containers do you use? When do you buy? means of transport?		
How long the milk stay prior sale? At what temperature? Containers?		
What kind of tests used to buy milk?		
What form of milk consumption (raw, boiled, boil with coffee, yogurt etc)? What is the rationale?		
Do you know any food poisoning/ GIT disturbance associated with drinking of raw milk? What are the symptoms?		
Did workers take training?		
How are frequently they visit physicians?		

Annex 8: RRA Checklist for the Identification dairy value chain in and around Debre-Zeit

1. Identify the respondents and establish if they own dairy farm

2. Identify dairy farming systems

3. Identify agents involved in the milk dairy value chain

4. Location of milk collection centers

5. What are the major health problems of dairy cows?

6. Occurrence of mastitis

7. Quantity of milk production, milk consumption pattern, source of milk to consumers and collection centers

7. Knowledge of food poisoning and SFP

Annex 9: Composition and preparation of media used for the study

- **BLOOD AGAR (OXOID ENGLAND)**

Typical formula (g/l):

'Lab-Lemco'powder…………………………………………10.0

Peptone…………………………………………………….10.0

Sodium chloride…………………………………………….5.0

Agar …………………………………………… ……...15.0

Final PH 7.3 $\pm$ 0.2 at 25 ^{0}C

Instructions:

Suspend 40g in 1L of demineralized (distilled) water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121 ^{O}C for 15 minutes. Cool to 45-50 ^{O}C and add 7% sterile defibrinated blood.

- **NUTRIENT AGAR (OXOID, ENGLAND)**

Compositions:

Typical formula (g/l):

'Lab-Lemco'powder……………………………………………..1.0

Yeast extract …………………………………………………..2.0

Peptone…………………………………………………………...5.0

Sodium chloride ……………………………………….......5.0

Agar …………………………………………………………15.0

Final PH 7.4 $\pm$ 0.2 at 25 ^{0}C

Instructions:

Suspend 28g in 1L of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121 ^{O}C for 15 minutes.

- ■ **MANNITOL SALT AGAR (OXOID, ENGLAND)**

Compositions:

Typical formula (g/l):

'Lab-Lemco'powder…………………………………………..1.0

Peptone………………………………………………………….10.0

Mannitol……………………………………………………...10.0

Sodium chloride ……………………………………….......75.0

Phenol Red ……………………………………………………0.025

Agar…………………………………………………………..15.0

Final PH 7.5 $\pm$ 0.2 at 25 ^{0}C

Instructions:

Suspend111g in 1L distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for15 minutes. Mix well before pouring into sterile Petri dishes.

- ■ **PURPLE AGAR BASE (DIFCO, FRANCE)**

Compositions:

Typical formula (g/l):

Proteose peptone …………………………………………..10.0

Beef extract…………………………………………... ………….1.0

Sodium chloride………………………………………………..5.0

Bromcresol Purple……………………………………………0.02

Agar…………………………………………………………..15.0

Final PH 6.8 $\pm$ 0.2 at 25 ^{0}C

Instructions:

Suspend 31g of the powder in 1L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to dissolve the powder. Autoclave at 121 ^{0}C for 15 minutes. When preparing 0.5-1% carbohydrate fermentation, dissolve 5-10g of the desired carbohydrate in the basal medium prior to sterilization by autoclaving.

- **BRAIN HEART INFUSION**

Ingredients
Pancreatic digest casein	14.5g
Agar	5g
Brain Heart Solids from infusion	8g
Peptic digest of Animal Tissue	5g
Sodium chloride	5g
Dextrose	2g
Sodium Phosphate Dibasic	2.5g
pH	7.4$\pm$0.2 at 25 ^{O}C
Distilled water	1liter

Instruction
Dissolve 52g in 1000 ml distilled water stir and dissolve completely and sterilized by autoclaving for 15 minutes at 121°C .Cool to room temperature before use.

9. CURRICULUM VITAE

A. PERSONAL INFORMATION

Full Name: Fanta Desissa Gutema

Sex: Male

Place of birth: Wollga, HorroGuduru, Guduru, Kubsa Kidame

Date of birth: June 13, 1981 GC

Martial status: Married

Language: Afan oromo, Amharic and English

Religion: Orthodox Christian

Nationality: Ethiopian

B. EDUCATIONAL BACKGROUND

Year(G.C.)	school	Class attended	Award
1987/88-1992/93	Kubsa kidame Elementary School	1-6	Ministry certificate
2008/09-2009/2010	Addis Ababa university	Postgraduate year I and II	Grade report; Masters in Tropical Veterinary public health will held July, 2010
2001/2002-2005/06	Addis Ababa university	2nd-6th	Doctor of Veterinary Medicine
2000/2001	Mekelle University	Freshman	Grade report
1997/98-1999/2000	Shambu senior and secondary school	11-12	Ethiopian School Leaving Certificate
1995/96-1996/97	Finchaa senior and secondary school	9-10	Certificate-Transcript
1993/94-1994/95	Embabo Tesfa junior and Elementary School	7-8	Ministry certificate

C. EDUCATIONAL QUALIFICATION

Doctorate degree in Veterinary Medicine (DVM)

3. Research out put

3.1. Campylobacteriosis: Its zoon tic importance (5th-seminar)

3.2. A study on the isolation and identification of bacterial flora from the upper respiratory tract of apparently healthy donkeys in central Ethiopia- published on International Journal of applied research in Veterinary Medicine, Volume 7, number 4, 2009, Pp181-189

3.3. Brucellosis: Host and agent diversity and its public health importance (postgraduate seminar)

3.4. Review on Epidemiology of Brucellosis in Ethiopia (in Press)

3.5. Quantitative Risk assessment of consuming raw milk contaminated with *Staphylococcus aureus* in and around Debre-Zeit (postgraduate thesis, ongoing)

4. **Skills**

4.1 . Computer (MS-word, MS-excel, MS-access)(Certificate)

4.2 . Management and leadership(Certificate)

5. **Experience**: Two years as full time lecturer in Wollega University and two years attending masters program in Veterinary Public Health while teaching undergraduate students at Addis Ababa University.

6. **References**

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Dr. Bojia Endebu (DVM), Donkey Health and Welfare project, FVM, Debre-Zeit

Dr. Teshale Sori (DVM), Department of Parasitology and Clinical Pathology, AAU, FVM, Debre-Zeit

Dr. Kohei MAKITA (DVM, PhD Veterinary Epidemiology, Department of Veterinary Hygiene and Environmental Sciences School of Veterinary Medicine Rakuno Gakuen University, Japan

10. SIGNED STATEMENT OF DECLARATION

I, the under signed, declare that this thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name: Fanta Desissa

Signature_____

Date of submission June 22, 2010

This thesis has been submitted for examination with my approval as a university advisor

Advisors:
 1. Name: Dr. Girma Zewde

    Signature: _____

2. Name: Akafete Teklu

    Signature: _____