

ADDIS ABABA UNIVERSITY
SCHOOL OF VETERINARY MEDICINE

**STUDY ON PREVALENCE OF *SALMONELLA* IN COTTAGE CHEESE AND CHEESE
MADE FROM PASTEURIZED MILK AND ITS PUBLIC HEALTH IMPORTANCE IN
DEBRE ZEIT, ETHIOPIA**

A thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial
Fulfillment of the Requirements of Masters Degree in

Veterinary Public Health

BY

YALEW TEFERA

BY

YALEW TEFERA

June, 2011

Debre Zeit, Ethiopia

ADDIS ABABA UNIVERSITY
SCHOOL OF VETERINARY MEDICINE

**STUDY ON PREVALENCE OF *SALMONELLA* IN COTTAGE CHEESE AND CHEESE
MADE FROM PASTEURIZED MILK AND ITS PUBLIC HEALTH IMPORTANCE IN
DEBRE ZEIT, ETHIOPIA**

Board of Examiners

Signatures

1. Dr. Nigussie Dugassa

School of public health sciences

**A thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial
Fulfillment of the Requirements of Masters Degree in**

3. Prof. Getachew Tishun

Veterinary Public Health

Akili Lema Institute of pathobiology

4. Dr. Genene Tefera

Institute of biodiversity conservation

BY

YALEW TEFERA

Academic Advisors

Dr. Akalite Tekle

(DVM, MSc, Assistant professor)

Professor Mahendra Pal

(BVM, MVS, PhD, DSc)

June, 2011

Debre Zeit, Ethiopia

**STUDY ON PREVALENCE OF *SALMONELLA* IN COTTAGE CHEESE AND CHEESE
MADE FROM PASTEURIZED MILK AND ITS PUBLIC HEALTH IMPORTANCE IN
DEBRE ZEIT, ETHIOPIA**

BY

YALEW TEFERA

Board of Examiners

Signature

1. **Dr. Nigusie Dugassa**

School of public health sciences

2. **Dr. Azage Tegege**

International livestock research institute

3. **Prof. Getachew Tilahun**

Aklilu Lema institute of pathobiology

4. **Dr. Genene Tefera**

Institute of biodiversity conservation

Academic Advisors

Dr. Akafite Teklu

(DVM, MSc, Assistant professor)

Professor Mahendra Pal

(BVSc, MVSc, PhD, DSc)

ACKNOWLEDGEMENTS

First and for most, I would like to thank the Almighty **GOD** for his innumerable tours, benevolence and mercy up on me.

My profound gratitude goes to my advisors Dr Akafete Teklu and Professor Mahendra Pal for their professional advice, kind cooperation with intellectual guidance, and devotion of time to make valuable corrections on this manuscript.

I am also very happy to express my deepest gratitude to Sorro Wereda Agricultural and Rural Development Office administrators who have done all what they can to help me attend my postgraduate study.

Finally, my gratitude should go to my parents and friends who have helped me in one or the other way.

1.5. Salmonella disease and pathogens	7
1.6. Food borne disease caused by microorganisms	9
1.7. Source of Salmonella	9
1.8. Salmonella in milk and milk products	12
1.9. Contamination of milk and milk products by Salmonella organism	13
1.10. Infective dose of Salmonella in human	15
1.11. Symptoms of Salmonella infection	16
1.11.1. Typhoid fever	17
1.11.2. Paratyphoid	17
1.11.3. Sheller's syndrome	17
1.12. Salmonella and Salmonellosis in Ethiopian condition	18
1.13. Public Health and economic impact	22
1.14. Control measures	25
2. MATERIALS AND METHODS	25
2.1. Study site	25
2.2. Study design	26
2.3. Sampling and study population	26

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	I
LIST OF TABLES	IV
LIST OF FIGURE	V
LIST OF ANNEXES.....	VI
ABBREVIATIONS.....	VII
ABSTRACT.....	IX
1. INTRODUCTION	1
2. LITERATURE REVIEW.....	4
2.1. Biology of <i>Salmonella</i>	4
2.2. Nomenclature of <i>Salmonella</i>	4
2.3. Morphology	6
2.4. Distribution of <i>Salmonella</i>	6
2.5. <i>Salmonella</i> : disease and pathogenesis.....	7
2.6. Food borne disease caused by microorganisms	9
2.7. Source of <i>Salmonella</i>	9
2.8. <i>Salmonella</i> in milk and milk products.....	12
2.9. Contamination of milk and milk products by <i>Salmonella</i> organism	13
2.10. Infective dose of <i>Salmonella</i> in human	15
2.11. Symptoms of <i>Salmonella</i> infection.....	16
2.11.1. Typhoid fever	17
2.11.2. Bacteremia.....	17
2.11.3. Reiter's syndrome.....	17
2.12. <i>Salmonella</i> and Salmonellosis in Ethiopian condition	18
2.13. Public Health and economic impact.....	22
2.14. Control measures	23
3. MATERIALS AND METHODS.....	25
3.1. Study site	25
3.2. Study design	26
3.3. Sampling and study population.....	26

3.4.	Methods.....	28
3.4.1.	Isolation.....	28
3.4.2.	Biochemical tests.....	29
3.4.3.	Culturing on chromogenic agar media.....	31
3.4.4.	Serotyping of <i>Salmonella</i> isolates	32
3.5.	Data analysis	32
4.	RESULTS.....	33
4.1.	Presence of growth on SS and/or XLD agar.....	33
4.2.	Storage time of cottage cheese and <i>Salmonella</i> positivity	33
4.3.	<i>Salmonella</i> isolates in cheese type, source and storage time	34
5.	DISCUSSION.....	36
6.	CONCLUSION AND RECOMMENDATIONS	40
7.	REFERENCES	41
8.	ANNEXES.....	56
9.	SIGNED DECLARATION SHEET	70

LIST OF TABLES

	PAGE
Table 1 Typical <i>Salmonella</i> and non typical bacteria grown on SSA and/or XLD media.....	33
Table 2 Storage time of the cottage cheese and <i>Salmonella</i> positivity.....	34
Table 3 <i>Salmonella</i> isolates in cheese type, source and storage time.....	35

LIST OF FIGURE

PAGE

Figure 1 map of the study site 26

Annex 2. Record sheets for laboratory analysis of samples for *Salmonella* 37

Annex 3. Composition and preparation of culture media used for the laboratory
 analysis of the study sample 38

Annex 4. Flow diagram for isolation and identification of *Salmonella* used in this study 63

Annex 5. Flow diagram for detection of *Salmonella* (Global Salm-Surv, 2003) 69

LIST OF ANNEXES

	PAGE
Annex 1. Sample collection record sheet for bacteriological analysis.....	56
Annex 2. Record sheets for laboratory analysis of samples for <i>Salmonella</i>	57
Annex 3. Compositions and preparation of culture media used for the laboratory analysis of the study sample.....	58
Annex 4. Flow diagram for isolation and identification of <i>Salmonella</i> used in this study.....	68
Annex 5. Flow diagram for detection of <i>Salmonella</i> (Global Salm-Surv, 2003).....	69

ABBREVIATIONS

AAU	Addis Ababa University
AIDS	Acquired Immunodeficiency Syndrome
CDC	Center for Disease Control
CIP	Clean In Place
CO ₂	Carbon Dioxide
°C	Degree Celsius
\$	Dollar
DNA	Deoxyribonucleic acid
DT	Definitive Type
FAO	Food and Agriculture Organization
FBD	Food Borne Disease
HACCP	Hazard Analysis Critical Control Point
H ₂ S	Hydrogen sulfide gas
HIV	Human Immuno Virus
HUS	Haemolytic Uremic Syndrome
ISO	International Standard Organization
KOH	Potassium Hydroxide
LDC	Lysine Decarboxylase
LIA	Lysine Iron Agar
MR-VP	Methyl red- Voges–Proskauer
NTS	Non Typhoidal <i>Salmonella</i>
PFGE	Pulsed field gel electrophoresis
QC	Quality Control
RV	Rappaport –Vassiliadis
SPI	<i>Salmonella</i> Pathogenesis Island
SSA	<i>Salmonella Shigella</i> Agar
TSI	Triple Sugar Iron
UK	United Kingdom
USA	United States of America

USDA	United States Drug Authority
VP	Voges–Proskauer
WHO	World Health Organization
XLD	Xylose Lysine Desoxycholate

Key words: Cheese, Cottage cheese, Public health, *Salmonella*, Storage time

ABSTRACT

This study was undertaken to determine the prevalence of *Salmonella* spp. in cottage cheese and cheese made from pasteurized milk in Debre Zeit and to assess the effect of storage time on isolation rate of *Salmonella* from cottage cheese. Samples for analysis were collected from Debre Zeit open market, Adada Dairy Processing Association and Genesis farms from September 2010 to April 2011. All together 384 cheeses (192 cottage and 192 cheese made from pasteurized milk) were collected and analyzed for the prevalence of *Salmonella*. Isolation and identification was performed according Global Salm-Surv (2003) and ISO 6579 (2002) standard methods. A total of 6 *Salmonella* species were recovered from 384 of the samples analyzed (1.6%). All the isolated *Salmonella* species were from cottage cheese (3.13%) and no *Salmonella* species were isolated from samples brought from Adaa Dairy Processing Association and Genesis farms. There was statistically significant ($P = 0.004$) difference in the prevalence of *Salmonella* between cottage cheese and cheese made from pasteurized milk. Statistically significant ($P = 0.027$) variation was also observed in the isolation rate of *Salmonella* in the storage time of cottage cheese in which higher prevalence was observed in cottage cheese stored for only one day. The results of the present study indicated that the temperature used for making cottage cheese in the study area is not sufficient to destroy *Salmonella* totally or contaminated after making the cheese. Therefore, it poses public health risk to the consumer. The pasteurization temperature in pasteurized milk used for cheese making in the milk processing plants is sufficient to kill *Salmonella*. This study also revealed that the fresh cottage cheese have higher *Salmonella* isolation rate than the stored cheese.

Key words: Cheese, Cottage cheese, Public health, *Salmonella*, Storage time

1. INTRODUCTION

Worldwide, food borne pathogens cause numerous sufferings and deaths. In Africa, Asia, and Latin America, there are about 1,000 million cases of gastroenteritis per year in children under the age of 5, which leads to 5 million deaths. In the US, there are an estimated 6 million cases with 350,000 hospitalizations and 9,000 deaths associated with food borne infections each year (Arun, 2008). Food borne illnesses are prevalent and common in all parts of the world, and the toll in terms of human life and suffering is enormous. Contaminated food contributes to 1.5 billion cases of diarrhea in children each year, resulting in more than three million premature deaths, according to the World Health Organization (WHO, 1999; CDC, 2009). Several outbreaks have been reported as a result of eating contaminated dairy food that may look taste and smell perfectly normal but is in fact contaminated with large harmful bacteria (CDC, 2009).

Salmonellae are among the most common bacterial food borne pathogens worldwide (Todd, 1997). *Salmonella* spp are also recognized as the leading causes of bacterial food borne diarrheal disease. The annual incidence of *Salmonella* infection is estimated to be about 16 per 100,000 populations causing 1.4 million estimated cases of food borne disease (FBD) each year in the United States alone (Voetsch *et al.*, 2004; FSM, 2005).

Despite the controls that have already been put into place, *Salmonella* infection arising from contaminated food continues to be an immense problem with millions of cases occurring annually throughout the world. In addition to the misery caused, financial loss is enormous (Global Salm-Surv, 2003).

Salmonellosis is considered as one of the most wide spread food borne zoonoses in industrialized as well as developing countries even though the incidence seems to vary between countries. It is usually difficult to evaluate the situation of salmonellosis in developing countries because of the very limited scope of studies and lack of coordinated epidemiological surveillance systems (Acha and Szyfres, 2001). Salmonellosis is the largest documented cause of food borne infections in France (Vaillant *et al.*, 2005).

Human salmonellosis is generally food borne and is contracted through consumption of contaminated food of animal origin such as meat, milk, poultry and eggs. Dairy products including cheese and ice cream were also implicated in the outbreak (Bell and Kyriakides, 2002; Pal, 2007).

Cheese is made in almost every country of the world and there exist more than 2000 varieties. Despite the large number of varieties cheese may be classified into different groups, namely ripened and unripened cheese, cheese with low or high fat content and cheese with soft or hard consistency. Unprocessed milk held at high ambient temperatures has a shelf-life from 2–3 hours up to 24 hours. Cheese, however, has a shelf-life from 4–5 days up to five years depending on the variety (O'Connor, 1993). *Ayib* is a traditional Ethiopian cottage cheese made from sour milk after the fat is removed by churning. It is an acidic product. *Ayib* is an important source of nutrients and serves as a staple diet. It may be consumed fresh as side dish, or it may be spiced with hot spices, salt and other herbs (Gonfa *et al.*, 2001). Most of the production of milk and various milk products in Ethiopia is generally a household process that usually takes place under unsanitary conditions (Ashenafi, 1989).

Unpasteurized dairy products have been shown to cause outbreaks of salmonellosis. In a study conducted on Tulum cheese, which is produced from raw milk and is one of the most popular semi-hard cheeses in Turkey to assess the presence of *L. monocytogenes* and *Salmonella* spp, 6 (2.4%) samples were positive for *Salmonella* Spp. (Hilal *et al.*, 2007). from the same country Tolga *et al.* (2010) has reported *Salmonella* spp in 1.9 percent of white cheese samples. In France, a nationwide outbreak of gastrointestinal illness due to *S. Muenster* occurred during March and April 2008. Twenty-five laboratory confirmed cases of *S. Muenster* were documented by telephone using a trawling questionnaire. Among the 21 interviewed cases, 16 reported consumption of goat's cheese in the days prior to symptoms (Van Cauteren *et al.*, 2009). *Salmonella* can be associated with many kinds of foods and the presence of *Salmonella* in ready-to-eat foods is considered significant regardless of the level of the contamination (Alakomi and Saarela, 2009).

Mohammed *et al.* (1996) have compared the detection of *Salmonellae* in Ethiopian cottage cheese (ayib) using different culture methods and he also isolated *Salmonella* from cheese in Awassa, Ethiopia. In another study Endrias and Poppe (2009) have reported a prevalence of 4(190) 2.1% *Salmonella* from cottage cheese and out of the four isolates one was resistant to antibiotics.

Except the above few studies in other area of the country, there are no works done on *Salmonella* in cheese and the public health risk due to consumption of cheese has not been known. Furthermore, in Ethiopia almost all cheese consumed at house hold level and sold in open market is produced traditionally from unpasteurized milk. Because of these the presence of food borne pathogens like *Salmonella* and others in cheese is predictable.

Therefore, the objectives of this study were

1. To determine the prevalence of *Salmonella* in Ethiopian cottage cheese sold in open market of Debre Zeit and pasteurized cheese form Adaa Dairy Processing Association and Genesis Farms in Debre Zeit.
2. To weigh against the public health risk of *Salmonella* infection from cottage cheese with cheese made from pasteurized milk.
3. To assess the effect of storage time on isolation rate of *Salmonella* from cottage cheese.

2. LITERATURE REVIEW

2.1. Biology of *Salmonella*

The genus *Salmonella* is a member of the *Enterobacteriaceae* family, and is a Gram-negative, nonspore-forming bacillus. Salmonellae are motile (except *Salmonella Pullorum* and *S. Gallinarum*) and express peritrichous flagella. They are facultative anaerobes that can grow in a temperature range of 5-45 °C with optimum temperature of 35-37 °C. They are able to grow at low pH and are generally sensitive to increased concentrations of salt. *Salmonella* forms long filamentous chains when grown at temperature extremes of 4-8 °C or at 44 °C and when grown at pH 4.4 or 9.4. All Salmonellae are facultative intracellular pathogen, considered pathogenic and can invade macrophages, dendritic and epithelial cells. The virulence genes responsible for invasion, survival, and extraintestinal spread are distributed in *Salmonella* pathogenicity islands (Arun, 2008).

2.2. Nomenclature of *Salmonella*

Salmonella is named after an American bacteriologist, Salmon, who first isolated *Salmonella choleraesuis* from porcine intestine in 1884 (Smith, 1894). *Salmonella* was initially divided into three distinct subspecies including *S. choleraesuis*, *S. typhosa* (*S. typhi*), and *S. Kauffmannii* containing all the other serovars (Su and Chiu, 2007). In 1966, Kauffman first proposed the one serotype-one species concept based on serological identification of somatic (O) and flagellar (H) antigens for nomenclature of *Salmonella* (Brenner *et al.*, 2000). In 1970, another proposal of *Salmonella* nomenclature was proposed to divide the serotypes into 8 subgenera based on the clinical roles, biochemical characteristics and genomic relevance of a strain (e.g., *S. kauffmannii* serovar typhi) (Su and Chiu 2007). In 1987, Le Minor and Popoff classified *Salmonella* as the single *Salmonella* species and seven subspecies on the basis of DNA-DNA hybridization experiments (Brenner *et al.*, 2000). Because there are several nomenclature systems for *Salmonella*, there is significant confusion in communication among scientists, health officials and even the public. Moreover, the recent development of genetic and molecular biology techniques is able to identify *Salmonella* more specifically, based on biochemical and serological

characteristics. Therefore, more systematic and uniform nomenclature for *Salmonella* is needed (Tindall *et al.*, 2005).

Salmonella nomenclature is complex and still evolving. Currently, the nomenclature system used at the Centers for Disease Control and Prevention (CDC) for the genus *Salmonella* is based on recommendations from the WHO Collaborating Centre. According to the CDC system, the genus *Salmonella* contains two species, *S. enteric*, which contains six subspecies (I: *enterica*, II: *salamae*, IIIa: *arizonae*, IIIb: *diarizonae*, IV: *houtenae* and VI: *indica*), the type species, and *S. bongori*. On March 18, 2005, a new species, “*Salmonella subterranean*” was validly approved by the Judicial Commission (Shelobolina *et al.*, 2004). There were 2541 described *Salmonella* serotypes as of 2007; approximately 60% belong to subspecies I. In the US, approximately 99% of *Salmonella* isolates from humans that are reported belong to subspecies I. The “top 10” most common serotypes from human specimens account for approximately 70% of all isolates reported in the US; the “top 100” serotypes account for about 98% of all isolates (CDC, 2008). The current nomenclature used by center for disease control and prevention (CDC) is based on the recommendations from the WHO collaborating centre and it adequately addresses the concern and requirements of clinical and public health microbiologists (Deb and Kapoor, 2005).

To avoid confusion between serotypes and species, the serotype name is not italicized and starts with a capital letter. When cited at the first time in a report, the genus name is given followed by the word “serotype” (or the abbreviation “ser.”) and then the serotype name, e.g., *Salmonella* serotype or ser. Choleraesuis, and *Salmonella* serotype or ser. Typhi. Afterward the name may be shortened with the genus name followed directly by the serotype name, e.g., *Salmonella* Choleraesuis or *S. Choleraesuis*, and *Salmonella* Typhi or *S. Typhi* (Popoff *et al.*, 2004).

2.3. Morphology

Salmonellae are Gram-negative, non spore forming, straight rods not exceeding 1.5 micrometers in width (Pal, 2007). They are facultative anaerobes usually motile by peritrichous flagella (European Commission, 2000a). Most Salmonellae form common fimbriae and most of them possess type-1 fimbriae associated with mannose-sensitive adhesive properties. These fimbriae are composed of fimbrillin subunits containing a high proportion of hydrophobic amino-acids (Old and Threlfall, 1998). *Salmonella* are routinely classified by serotype on the basis of expression of three surface antigens, the somatic O antigen, the flagella H1 and H2 antigens and the capsular Vi antigen, according to the Kauffmann-White scheme (Scott *et al.*, 2002). The absence of flagella may consequently affect complete identification of the serotype; *Salmonella enterica* serovar Typhimurium exhibits morphological differences dependent on the peptone constituents of the culture medium however, in media containing soy-based peptone as the primary nutrient, *Salmonella* displays a normal flagellated morphology (Victoria *et al.*, 2006).

2.4. Distribution of *Salmonella*

The primary habitat of *Salmonella* spp. is the intestinal tract of animals such as birds, reptiles, farm animals, humans, and occasionally insects. Although their primary habitat is the intestinal tract, they may be found in other parts of the body from time to time. As intestinal forms, the organisms are excreted in feces from which they may be transmitted by insects and other living creatures to a large number of places. As intestinal forms, they may also be found in water, especially polluted water. When polluted water and foods that have been contaminated by insects or by other means are consumed by humans and other animals, these organisms are once again shed through fecal matter with a continuation of the cycle. The augmentation of this cycle through the international shipment of animal products and feeds is in large part responsible for the worldwide distribution of salmonellosis and its consequent problems (James and Detroit, 2000).

Salmonellae have been found in commercially prepared and packaged foods with 17 of 247 products examined being positive. Among the contaminated foods were cake mixes, cookie

dough, dinner rolls, and cornbread mixes. These organisms have been found in coconut meal, salad dressing, mayonnaise, milk, and many other foods (James and Detroit, 2000).

2.5. *Salmonella*: disease and pathogenesis

Salmonella are well-known pathogens, highly adaptive and potentially pathogenic for humans and/or animals. *Salmonella* infections are capable of producing serious infections that are often food borne and present as gastroenteritis. However, a small percentage of these infections may become invasive and result in bacteremia and serious extra intestinal disease (Fluit, 2005). The main reservoirs for non-typhoidal *Salmonella* are animals such as poultry, livestock, pets and reptiles. *Salmonella* Typhi and *Salmonella* Paratyphi colonize only in humans, so they can be acquired only from close contact with a person who has typhoid fever, from a chronic carrier, or from water or food contaminated by human feces (CIDRAP, 2006).

While certain serovars of *Salmonella enterica* cause disease in humans and a variety of animals, other serovars are highly restricted to a specific host. *Salmonella* infections range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhea and vomiting, to typhoid fever, a life threatening infection (Hensel, 2004). The outcome of *Salmonella* infections is determined by the host and the status of the bacterium. Whereas, age, genetic and environmental factors mainly determine the status of the host, the status of the bacterium is determined by so-called virulence factors (Alphons *et al.*, 2005).

Serotypes adapted to man, such as *Salmonella* Typhi and *Salmonella* Paratyphi, usually cause severe diseases in humans as a septicaemic typhoidic syndrome (enteric fever). These serotypes are not usually pathogenic to animals. Serotypes that are highly adapted to animal hosts, such as *Salmonella* Gallinarum (poultry) or *Salmonella* Abortus-ovis (sheep), usually produce very mild symptoms in man. However, *Salmonella* Choleraesuis which has the pig as a primary host also causes severe systemic illness. In the same way, *Salmonella* Dublin, which has a preference for bovines, is primarily responsible for the systemic form of Salmonellosis. In young calves this disease causes high mortality, and in adult cattle it results in fever, reduced milk yield, diarrhea, abortion, and occasionally death. Ubiquitous serotypes, such as *Salmonella* Enteritidis or *Salmonella* Typhimurium, which affect both man and animals, generally cause gastrointestinal

infections usually less severe than enteric fever. However, they also have the capacity to produce typhoid-like infections in mice and in humans, or asymptomatic intestinal colonization in chickens (Velge *et al.*, 2005).

Salmonella avoid host defense in the stomach and reach the intestines, (CIDRAP, 2006) and the bacteria interact with the non-phagocytic cells such as the epithelial cells of the intestinal mucosa (Hensel, 2004). They adhere to the intestinal epithelial cells by adhesive structures (fimbriae) that promote binding and invade epithelial cells to provoke gastroenteritis. The organisms have virulence factors such as virulence-plasmids, toxins, fimbriae and flagella that help in establishing an infection (Alphons *et al.*, 2005).

The mechanism of pathogenesis has been described in the following steps (Mi-Kyung, 2009)

Bacterial mediated endocytosis: A highly coordinated series of interactions between proteins released by *Salmonellae* and proteins of the host cell causes host cellular surface membrane ruffling and engulfment of bacteria in cellular vacuoles.

Neutrophil recruitment and migration: *Salmonellae* associated with gastroenteritis induce a secretory response in intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen.

Epithelial cell cytokine secretion: In tissue culture models of *Salmonella* Enteritidis, translocation of SPI-1 (*Salmonella* pathogenesis Island one) proteins into intestinal epithelial cells leads to synthesis and polarized secretion of inflammatory mediators and neutrophil chemo attractants.

Fluid and electrolyte secretion: Several translocated SPI-1 proteins contribute to intestinal inflammation and fluid secretion. Intestinal inflammation probably contributes to fluid secretion and diarrhea by disrupting the epithelial barrier and increasing water flux by an exudative mechanism. Innate immune system activation also contributes to intestinal inflammation.

Systemic infection: *Salmonella* Typhi invades macrophages and the migration of infected macrophages to reticuloendothelial organs via the lymphatic system and blood produces systemic illness with less diarrhea.

2.6. Food borne disease caused by microorganisms

Food borne disease is a public health problem which comprises a broad group of illnesses. Among them, gastroenteritis is the most frequent clinical syndrome which can be attributed to a wide range of microorganisms, including bacteria, viruses and parasites. Usually, the incubation period is short, from 1-2 days to 7 days. Different degrees in severity are observed, from a mild disease which does not require medical treatment to the more serious illness requiring hospitalization, long-term disability and/or death (hospitalization rates from 0.6% to 29% and case-fatality rates up to 2.5% in the USA) (Mead *et al.*, 1999). The outcome of exposure to food borne diarrhoeal pathogens depends on a number of host factors including pre-existing immunity, the ability to elicit an immune response, nutrition, age and non-specific host factors. As a result, the incidence, the severity and the lethality of food borne diarrhoea is much higher in some particularly vulnerable segments of the population, including children under five years of age, pregnant women, immunocompromised people (patients undergoing organ transplantation or cancer chemotherapy, AIDS) several authors have estimated that chronic sequelae (long-term complications) may occur in 2% to 3% of all FBD (Lindsay, 1997).

2.7. Source of *Salmonella*

Human salmonellosis is generally food borne and is contracted through consumption of contaminated food of animal origin such as meat, milk, poultry, and eggs. Dairy products including cheese and ice cream were also implicated in the outbreak (Bell and Kyriakides, 2002).

The primary sources of *Salmonella* are the gastrointestinal tract of humans, domestic and wild animals, birds and rodents. Consequently, they are widespread in the natural environment including soil and waters in which they do not usually multiply significantly but may survive for long periods, i.e. many months in soil and dried animal faeces. *Salmonella* serotypes have been reported in a variety of fresh produce products. A survey of fresh products imported from 21 countries into the USA, carried out by the US Food and Drug Administration in 1999, found 3.5% (35/1003) samples positive for *Salmonella* (FDA, 2001).

Transmission routes to humans are mainly between humans, from animals via the food supply and from water and the environment. Foods contaminated by *Salmonella* usually look and smell normal; it is therefore essential that every effort is made by all those in the food production and supply industries to exclude the organism from foods wherever possible (Ioannis, 2009).

In some cases, salmonellosis may be associated with uncommon sources. It is frequently the result of factors including the ubiquitous distribution of *Salmonella* serovars, usage of contaminated ingredients, incorrect cleaning or disinfection of equipment in the food industry, and infected employees working in restaurant kitchens (Freitas *et al.*, 2010).

On occasion, multiple serotypes of *Salmonella* have been found associated with food raw materials implicated in outbreaks, e.g. 11 strains were identified in paprika powder including *S. Saintpaul*, *S. Javiana*, *S. Rubislaw*, *S. Florian*, *S. Loenga* and six un-named strains (Lehmacher *et al.*, 1995). Mung bean seeds, cocoa beans and coconut have also been variously reported to contain more than one serotype of *Salmonella*. O'Mahony *et al.* (1990) reported five serotypes isolated from bean sprouts.

In the USA, an outbreak caused by a *Salmonella* strain with a specific Pulsed field gel electrophoresis (PFGE) pattern occurred during January 1- December 31, 2007. In this outbreak, a total of 401 cases from 41 states were identified and 32% of ill persons were hospitalized. A case-control study indicated that illness was associated with consumption of not-ready-to-eat pot pies. Later, the outbreak strain was isolated from 13 samples of unopened pot pies collected from the homes of patients (CDC, 2008). Moreover, in November 2006, public health officials of CDC and state health departments detected an increase in the reported incidence of isolates of *S. Tennessee*. In a multi-state case-control study conducted during February 5-13, 2007, illness was demonstrated to be strongly associated with the consumption of two brands of peanut butter produced at the same process plant. A total of 628 persons infected with this outbreak strain of *S. Tennessee* had been reported from 47 states during 2006-2007 (CDC, 2007a). Later, on November 25, 2008, an epidemiological investigation began due to an increase of *S. Typhimurium* isolates that shared the same PFGE patterns. Up to January 2009, 529 persons from 43 states from the USA and one person from Canada had been reported infected with this strain. A total of 116 patients were hospitalized, and the infection might have contributed to eight

deaths. Sequential case-control studies indicated significant associations between illness and consumption of peanut butter, and specific brands of peanut butter biscuits. Epidemiological and laboratory findings indicated that peanut butter and peanut paste produced at one plant were the sources of the outbreak. These products were also ingredients in many foods produced and distributed by other companies. This outbreak highlights the complexities of "ingredient-driven" outbreaks and the importance of rapid outbreak detection and investigation (CDC, 2009).

On November 15, 2006, in the USA a case of salmonellosis in an employee of a poultry vaccine facility was notified. When a second case of salmonellosis in another employee at the same facility was reported on November 25, an outbreak investigation was initiated. Results of that investigation suggested that employees of the facility became ill during a 1-month period from exposure to a strain of *S. Enteritidis* that was used in vaccine production. Infection was thought to have resulted from environmental contamination after the spill of a liquid containing a high concentration of *S. Enteritidis*. As a result, it was recommended that the facility improve its infection-control procedures to better protect workers. This outbreak highlights occupational risks that can be associated with the manufacture of veterinary biologicals derived from human pathogens (CDC, 2007b).

During October and November 2006, the Spanish National Reference Laboratory reported a series of isolations of *S. Kottbus* on the island of Gran Canaria. A matched case-control study was conducted and it showed that the ingestion of a brand of bottle water was related to outbreak. Microbiological and environmental analysis detected *S. Kottbus* in bottles randomly selected from markets, and also in the local factory where the water was bottled. A carrier pigeon loft was found near one water reservoir of the local factory and it could be verified that pigeons frequented this reservoir. Moreover, *Salmonella* spp. was detected in the pigeons, suggesting that they could be contaminating the water reservoir (Palmera-Suárez *et al.*, 2007).

In 2002, an outbreak of *S. Enteritidis* phage-type 8 occurred at a convention center in Dallas, Texas, USA and continued for 6 weeks. From March to April, the hotel kitchen implicated catered for 41 multi-day conferences attended by 9,790 persons. A total of 617 illness reports from residents of 46 states were received. The epidemiological studies showed that sauces or items served with sauces were implicated in outbreak. Eleven food service employees, including

one who prepared sauces, had stool cultures that yielded *S. Enteritidis* phage-type 8. Transmission ended with implementation of policies to screen food handlers and exclude those whose stool cultures yielded *S. Enteritidis* (Beatty *et al.*, 2009).

2.8. *Salmonella* in milk and milk products

The prevalence of *Salmonella* species in bulk tank milk has been reported to range from 1% to almost 9%. A high percentage of human salmonellosis occurs through consumption of raw milk or dairy products manufactured with raw milk (CDC, 2002, 2003).

Several *Salmonella* serotypes have been isolated from clinically ill cattle. *Salmonella enterica* serotype Typhimurium definitive type 104 (DT 104) is of particular concern to animal and public health agencies due to its multiple antimicrobial resistance (Besser *et al.*, 2000). Inline milk filters from farms located in central, east, north, and west regions of New York State were evaluated to determine the prevalence of *Salmonella* in New York dairy herds. Six of 404 (1.5%) milk filters were positive for *Salmonella* spp. From these isolates, one was confirmed as *S. Typhimurium* DT 104 (Hassan *et al.*, 2000).

In another study conducted on 12 dairy farms from Minnesota, Michigan, New York, and Wisconsin where fecal, bulk tank milk, and environmental samples were analyzed for *Salmonella*, it was found that 1.1% of bulk tank milk ($n=91$) and 12.6% of in-line milk filter samples ($n=87$) were positive for *Salmonella* spp. (Warnick *et al.*, 2003).

Recently, there has been an increase in infections caused by *Salmonella enterica* serotype Newport (CDC, 2002; Weir *et al.*, 2004). These infections can be severe. An increasing number of *S. Newport* isolates have been shown to be resistant to 9 or more antimicrobial agents (Zhao *et al.*, 2003). Risk factors that may be associated with *S. Newport* infection in humans include direct exposure to dairy farms, ingestion of raw milk and cheese made from unpasteurized raw milk, and consumption of raw or undercooked ground beef (Gupta *et al.*, 2003). Results of the recent National Animal Health Monitoring System Dairy 2002 Survey reported by Van Kessel *et al.* (2004) indicated that 2.6% of bulk tank milk samples were culture-positive for *Salmonella*, and four of 22 isolates were *S. Newport*.

2.9. Contamination of milk and milk products by *Salmonella* organism

In December 1996, an increase in the number of isolations of an uncommon *Salmonella* serotype was noted in the Northern and Yorkshire health region of England. A case control study of 15 cases indicated a strong association with the consumption of a single food product (Anon, 1996a). The product was a mild, colored, Cheddar cheese, manufactured from pasteurized milk and sold in bulk packs (Anon, 1996b).

Cheese, of both pasteurized and unpasteurized varieties, has been implicated in a large number of outbreaks of salmonellosis. In many cases it is the use of unpasteurized milk, either intentionally or unintentionally that appears to have been the most significant factor in the cause of outbreaks.

In 1984 a large outbreak of salmonellosis caused by *S. Typhimurium* PT10, affecting more than 1500 people, was reported in Canada (D'Aoust *et al.*, 1985) due to the consumption of Canadian cheddar cheese made from pasteurized or thermised (heat treated but not fully pasteurized) milk (D'Aoust, 1989). In 1989, imported Irish soft cheese made from unpasteurized milk was responsible for salmonellosis affecting 42 people in England and Wales (Maguire *et al.*, 1992). The implicated product in this outbreak was supposed to be made from pasteurized milk. Therefore, whilst raw milk quality would still have been important, the critical control in relation to safety would have been the pasteurization process. Pasteurization, if carried out properly, is known to be capable of destroying *Salmonella*, if present in the raw milk (D'Aoust *et al.*, 1987).

A common reason why pasteurized milk becomes contaminated with unpasteurized milk is pasteurizer failure coupled with an ineffective flow diversion system to prevent improperly pasteurized milk continuing in forward flow. This may occur due to failure of the flow diversion valve or, more commonly, due to manual override of flow diversion. Many older pasteurizers have a manual override system, principally to allow forward flow during clean in place (CIP) cycles. Manual override of the pasteurizer during milk pasteurization must never occur during the milk pasteurization operation. In small factories, operatives often have multiple duties but it is essential that the operating pasteurizer remains under constant supervision by properly trained staff because flow diversion may require operative intervention, e.g. switching the raw milk pump off to prevent overflowing of the diversion tank when milk is sent into it. Contamination

of pasteurized milk may also occur through improperly maintained pasteurizer plates, with raw milk entering the pasteurized milk stream through cracks/pinholes in the pasteurizer plates (Bell and Kyriakides, 2002).

Cheese, of both pasteurized and unpasteurized varieties, has been implicated in a large number of outbreaks of salmonellosis. In many cases it is the use of unpasteurized milk, either intentionally or unintentionally that appears to have been the most significant factor in the cause of outbreaks. In 1984 a large outbreak of salmonellosis caused by *S. Typhimurium* PT10, affecting more than 1500 people, was reported in Canada (D'Aoust *et al.*, 1985) due to the consumption of Canadian cheddar cheese made from pasteurised or thermised (heat treated but not fully pasteurised) milk (D'Aoust, 1989). In 1989, imported Irish soft cheese made from unpasteurized milk was responsible for salmonellosis affecting 42 people in England and Wales (Maguire *et al.*, 1992). The outbreak strain was *S. Dublin* and this was isolated from samples of cheese taken from the manufacturer's premises. The manufacturer subsequently modified the process to pasteurize milk for the production of the cheese. Cody *et al.* (1999) reported two outbreaks of *S. Typhimurium* DT104 affecting 31 and 79 people, respectively, following the consumption of unpasteurized milk and Mexican-style cheese in northern California. Altekruse *et al.* (1998) reviewed cheese-associated outbreaks of illness in the USA between 1973 and 1992 and reported that three outbreaks had been caused by *Salmonella*.

Recently, an outbreak of *S. Typhimurium* illness affecting 113 people was reported in France due to the consumption of a soft ripened cheese made from raw milk. *Salmonella* Typhimurium was isolated from products retrieved from patients' homes and the home of one individual not suffering illness (De Valk *et al.*, 2000).

It is vitally important that all persons involved in critical aspects of food process control are thoroughly trained and understand the process, reasons for and methods of control. Also, they must be fully aware of their responsibilities and completely understand the implications of any deviation from proper practice. There are several other stages during the manufacturing process where cheese can become contaminated but the most critical point for pasteurized milk cheese is the milk pasteurization stage. In addition to control of effective pasteurization times and temperature, pasteurizers should be routinely tested for leakage between the plates. Post-process

contamination due to poor segregation of raw and pasteurized milk or personnel with access to both sides of the factory are also important elements to consider in the control of these products. Environmental hygiene and effective cleaning and sanitization of all equipment including the cheese vats, moulds and maturation rooms is also critical to avoiding contamination entering at these points of the process (Bell and Kyriakides, 2002).

In the manufacture of cheese made from unpasteurized milk, milking hygiene is probably the most critical stage contributing to the overall safety of the product. As such, sources of milk for the production of unpasteurized milk cheese should be under regular review in relation to adherence to high standards of hygiene. This should be supported by regular microbiological testing of the ex-farm milk for the presence of pathogens and indicators of faecal contamination such as *E. coli* (anon, 1997).

2.10. Infective dose of *Salmonella* in human

The infective dose for causing food borne salmonellosis in humans was, for decades, believed to be high, i.e. 100000 to one million cells, but a number of outbreaks have now occurred in which the infective dose was found to be low, e.g. < 10-100 cells. This is especially the case where products containing a high fat level are involved, e.g. chocolate, cheese and salami (Cheddar cheese Heidelberg 100-500 cells (Fontaine *et al.*, 1980), Cheddar cheese Typhimurium PT10 1 (< 1-10) (D'Aoust *et al.*, 1985; D'Aoust, 1989), Chocolate balls Eastbourne < 100 (D'Aoust *et al.*, 1975) and Chocolate bar Napoli < 50 (Greenwood and Hooper, 1983), and where the consumers are children or elderly people. Realizing that such a low number of cells of *Salmonella* can be capable of causing illness serves to underline the importance of all the measures required to be taken to minimize the incidence and level of the organism at all stages of the food chain. Also, in order to provide reliable information, the microbiological test methods used to support hazard analysis, critical control point (HACCP) based food production safety and quality systems must be carefully selected and operated to ensure that they are suitable for detecting potentially low numbers in the sample types under examination.

The infectious dose of *Salmonellae* can vary, depending on the bacterial strain ingested as well as on the immuno-competence of individuals. For serotypes not presenting particular adaptations

to an animal host, experimental studies showed that between 10⁵ to 10⁷ bacteria were required to establish an infection (McCullough and Eisele, 1951). However, data from outbreaks of foodborne diseases indicate that infections can be caused by ingestion of as few as 10-45 cells (D'Aoust *et al.*, 1985; Lehmacher *et al.*, 1995). It has repeatedly been reported that the infectious dose is lower when *Salmonellae* are present in food with a high content of fat or protein, substances which protect bacterial cells against the low pH of gastric juices and where the consumers are children or elderly people (D'Aoust *et al.*, 1975; Blaser and Newman, 1982).

Some serotypes have developed a high host adaptation such as *S. Pullorum* and *S. Gallinarum* in poultry, *S. Dublin* in cattle, *S. Abortus-ovis* in sheep and *S. Cholerae-suis* in swine. Animals infected with non-host adapted *Salmonellae* are usually asymptomatic carriers, although some may exhibit clinical signs of low to moderate severity. Carriers that have not been recognised as such present a risk of shedding *Salmonellae* into the environment. According to a document from European commission, any serotype that is not host-adapted is considered capable of causing gastrointestinal illness of varying severity in humans (European commission, 2003).

2.11. Symptoms of *Salmonella* infection

Typically, non-typhoidal *Salmonella* produces a self-limiting febrile gastrointestinal illness that is indistinguishable from that caused by other bacterial enteric pathogens. Dehydration is the principal clinical concern. The incubation period – the time between ingestion of *Salmonella* bacteria and the onset of illness – varies from six to 72 hours (MMWR Recomm Rep, 2001; Mayo Clinic, 2007).

Salmonella can cause three different kinds of illness: gastroenteritis, typhoid fever, and bacteremia. Symptoms of *Salmonella* gastroenteritis include diarrhea, abdominal cramps, fever, nausea, and/or vomiting (Pal, 2007). In mild cases diarrhea may be non-bloody, occur several times per day, and not be very voluminous; in severe cases it may be frequent, bloody and/or mucoid, and of high volume.

Fever generally occurs in the 100°F to 102°F (38°C to 39°C) range. Vomiting is less common than diarrhea. Headaches, myalgias (muscle pain), and arthralgias (joint pain) are often reported

as well. Whereas the diarrhea typically lasts 24 to 72 hours, patients often report fatigue and other nonspecific symptoms lasting 7 days or longer (Mayo Clinic, 2007).

Salmonella infection itself usually isn't life-threatening. However, in certain people especially infants and young children, older adults, transplant recipients, pregnant women and people with weakened immune systems, the development of complications can be dangerous (Mayo Clinic, 2011).

2.11.1. Typhoid fever

Typhoid fever, also known as enteric fever, is caused by *Salmonella* serotype Typhi. The onset of symptoms usually occurs between 5 and 21 days after ingestion of *Salmonella* Typhi bacteria. Symptoms may include constipation, cough, sore throat, headache, and a rash on the infected individual's chest, as well as the slowing of the heartbeat and enlargement of the liver and spleen (Mayo Clinic, 2007).

2.11.2. Bacteremia

Two to eight percent of nontyphoid *Salmonella* (NTS) infections are associated with bacteremia, and are not always preceded by gastroenteritis. Risk factors for NTS bacteremia include immunocompromise (including HIV, malignancy, chemotherapy, steroid therapy) and extremes of age (< 3 month and greater than 50 years old). Risk factors are not apparent in up to one third of cases of NTS bacteremia. Extra intestinal focal infections (e.g. arthritis, meningitis, pneumonia) occur in 5-10% of those with bacteremia (Matheson, 2010).

2.11.3. Reiter's syndrome

Reiter's syndrome which includes and is sometimes referred to as "reactive arthritis" is an uncommon, but debilitating, result of a *Salmonella* infection. Reiter's syndrome is a disorder that causes at least two of three seemingly unrelated symptoms: reactive arthritis, eye irritation, and urinary tract infection (Hill and Lillicrap, 2003). The reactive arthritis associated with Reiter's develops when a person eats food that has been tainted with bacteria. Reactive arthritis is characterized by the inflammation of one or more joints following an infection localized in another portion of the body, commonly the gastrointestinal tract. The symptoms of Reiter's

Syndrome usually occur between one and three weeks after the infection (http://www.medpedia.com/news_analysis/148-Marlerblog/entries/16057-Salmonella-Montevideo-Outbreak-in-the-Offing).

The three most common symptoms of Reiter's syndrome are arthritis, eye irritation, and urinary tract symptoms. The arthritis associated with Reiter's syndrome typically affects the knees, ankles, and feet, causing pain and swelling. Wrists, fingers and other joints can be affected, though with less frequency. Patients with Reiter's syndrome commonly develop inflammation where the tendon attaches to the bone, a condition called enthesopathy. Some patients with Reiter's syndrome also develop heel spurs, bony growths in the heel that cause chronic or long-lasting foot pain. Arthritis from Reiter's syndrome can also affect the joints of the back and cause spondylitis, inflammation of the vertebrae in the spinal column. The duration of reactive arthritis symptoms can vary greatly. Most of the literature suggests that the majority of patients recover within a year. The condition, can, however, be permanent. One study found nearly 50 percent of patients with post dysenteric reactive arthritis continued to have symptoms roughly one year after onset (Inman *et al.*, 1998).

The involvement of the eye in Reiter's syndrome is most commonly manifested as conjunctivitis, inflammation of the mucous membrane that covers the eyeball, or uveitis, an inflammation of the inner eye. Conjunctivitis and uveitis can cause redness of the eyes, eye pain and irritation, and blurred vision. The third site for Reiter's syndrome symptoms is the urogenital tract. This includes the prostate, urethra, and penis in men and the fallopian tubes, uterus, and vagina in women. Men may notice an increased need to urinate, a burning sensation when urinating, and a discharge from the penis. Some men also develop prostatitis. Symptoms of prostatitis include fever, chills, increased need to urinate, and a burning sensation when urinating (http://www.about-salmonella.com/salmonella_symptoms_risks).

2.12. *Salmonella* and Salmonellosis in Ethiopian condition

In Ethiopia, like other developing countries, it is difficult to evaluate the situation of salmonellosis. This is mainly because of the very limited scope of studies, lack of coordinated epidemiological surveillance system and inadequacy of laboratory facilities for culture. In

addition, under reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis in some countries, including Ethiopia (Beyene *et al.*, 2008).

The first comprehensive study on the serogroup of *Salmonella* species in Ethiopia was conducted by Gedebeu and Tassew (1981) in Addis Ababa. In this study, a total of 165 *Salmonella* isolates, collected from various hospitals in Addis Ababa from 1975-80, of which 131 (79.4%) were from blood, 18 (11%) from stool and the rest 16 (9.7%) were from other specimens. Of the 165 isolates, 7 were group A, 16 B, 1 E, 17 C and the remaining 124 were *S. Typhi*. Since most of the specimens available were blood collected from inpatients with suspected typhoid fever, this may not reflect the true isolation rate of non-typhoidal *Salmonellae* from stool.

From 1974 to 1981 Gebre-Yohannes conducted a study to identify the prevalent serotypes and their susceptibility to drugs in Addis Ababa (Gebre-Yohannes, 1985). He studied 216 *Salmonella* strains which were predominantly isolated from adult patients referred from different hospitals to the Central Laboratory and Research Institute, Addis Ababa between January 1974 and October 1981. Serogroups B, C, D, E and A were isolated at frequencies of 20.4%, 19.4%, 6.9%, 2.8% and 1.9% respectively. Out of the 216 *Salmonella* strains, 48.6% were *S. Typhi*. This base-line study identified the existence of 26 different serotypes among the 216 *Salmonella* isolates. In his findings, *S. Concord* (12.5%) and *S. Typhimurium* (11.1%) predominates the non-Typhi isolates. The high isolation rate of *S. Concord* in Ethiopia was unusual in contrast to high prevalence of *S. Typhimurium* elsewhere (Lepage *et al.*, 1984).

However in a recent study conducted following the report of Multidrug-resistant *Salmonella* serovar Concord infections in children adopted from Ethiopia showed the higher prevalence of this serovar. Hendriksen and his colleagues interviewed patients, characterized the isolates, and gathered information about adoptions from Ethiopia to assess public health implications. They also visited orphanages in Ethiopia. *Salmonella* Concord was isolated from 78 persons from 2003 to 2007. Adoption status was known for 44 patients 3 years of age; 98% were adopted from Ethiopia (Hendriksen *et al.*, 2009).

Salmonella can cause food poisoning in humans, as demonstrated by Aseffa and his colleagues (1994), who isolated, between December and January 1992, nine *S. Newport* from an outbreak of

food poisoning among college students in Gondar, believed to be due to contaminated eggs. In their investigation, *S. Newport* was isolated from the stool of six students and three food handlers, none of the food source remained available for analysis.

Asrat (2008) reported that among the 37 *Salmonella* strains collected during the period of 1992-1993 in Tikur Anbesa Hospital, the most common serogroup was group B (81.1%), followed by group D (*S. Typhi*) (10.8%) and group C (8.1%).

Salmonella serotypes isolated from food animals and foods of animal origin also showed high level of resistance against different antimicrobial agents, for example, 31.8% of *Salmonella* isolates from sheep and goat (Molla *et al.*, 2006), 44.4 % of isolates from camels, (Molla *et al.*, 2004), 52% isolates from cattle and 87.2% isolates from minced beef and chicken (Molla *et al.*, 1999) were resistant to one or more antimicrobials commonly used to treat both human and animal infections.

Salmonellosis caused by drug resistant *Salmonella* strains is a major health problem, especially among children in developing countries like Ethiopia. A study report by Mache (2002), to determine the prevalent *Salmonella* serogroups and resistance pattern of the isolates to commonly used antibiotics among children in Jimma, South West Ethiopia, showed that, all the isolates were 100% susceptible for polymyxin B and gentamicin except one strain in serogroup D and B respectively, while 91% of the isolates were susceptible to nalidixic acid. The susceptibility of all strains in each serogroup to ampicillin and tetracycline was below 48%. Between 52.5%, to 74.6% of the isolates were sensitive to the other antibiotics.

On the other hand similar study was done aimed at isolation of potential enteric bacterial pathogens from the stool of HIV-infected and HIV-non infected patients and their antimicrobial susceptibility patterns in Jimma, Hospital, South west Ethiopia (Awole *et al.*, 2002). This study showed that, 90% of *Salmonella* isolates from both patient groups were resistant to doxycycline, amoxicillin, carbenicillin, and streptomycin. The most extensive drug resistance pattern encountered was to amoxyciline, cephalothin, chloramphenicol, trimethoprim-sulfamethoxazole and doxycycline.

Wolday (1998) has conducted a study on the increasing incidence of multidrug resistant *Salmonella* in Ethiopia. He studied 110 *Salmonella* isolates (48 *S. Typhi* and 62 non-Typhi *Salmonella*) collected between 1993 and 1996 from hospitalized patients in Tikur Anbessa Hospital, Addis Ababa. He tested the isolate against commonly used drugs: ampicillin, carbenicillin cephalothin, chloramphenicol, gentamicin, kanamycin, tetracycline, amikacin, cefuroxime and trimethoprim/sulphamethoxazole (Wolday, 1998). The study showed that thirty-five (31.8%) isolates were susceptible to all of the antibiotics tested, while 29 (26.4%) were resistant to only one drug (most commonly trimethoprim/ sulphamethoxazole) and five (4.5%) were resistant to two drugs (most commonly tetracycline and trimethoprim/ sulphamethoxazole). The remaining 41(37.3%) were resistant to three or more drugs. Cefuroxime was the only drug to which all strains were susceptible. The majority of non- Typhi *Salmonella* were resistant to chloramphenicol (61.7%), ampicillin (59.7%), carbenicillin (53.2%) and trimethoprim-sulphamethoxazole (51.6%), while approximately 30% were resistant to each of gentamicin, kanamycin, cephalothin and tetracycline.

A very recent study was conducted by Beyene and his colleagues to determine the etiology of febrile and diarrhoeic illness in Ethiopian children focusing on *Salmonella*. 85 of the 113 isolates of *Salmonella* were *Salmonella* concord and Multi-drug resistant *S. Concord* was the major cause of salmonellosis in two regions of Ethiopia according to this study (Beyene *et al.*, 2011).

In a review made by Beyene and his colleagues (2008) from the reports of salmonellosis by investigators in different parts of Ethiopia and in particular focusing on the levels of typhoid fever, there have been seven studies which diagnosed typhoid fever by laboratory culture and there was no coordinated epidemiological surveillance. All conducted research and reports from different health institutions in Ethiopia indicate that typhoid fever was still a common problem up to the most recent study in 2000 and that the extensive use of first-line drugs has led to the development of multiple drug resistance. In the sites covered by this review, the total number of published cases of typhoid fever dropped over time reflecting the decline in research capacity in the country. Data on the proportion of patients infected by different serovars of *Salmonella* suggest that the non-Typhi serovars of *Salmonella* are increasing. The published evidence suggests that typhoid fever is a current public health problem in Ethiopia, although population based surveys based on good microbiological diagnosis are urgently needed.

2.13. Public Health and economic impact

While it is recognized that the prevalence of food borne illnesses in developing countries is considerable, in most countries there is limited data through which the incidence of particular diseases and trends can be assessed over time (Henson, 2003b). The growing movement of people, live animals, and food products across borders; rapid urbanization in developing countries; increasing numbers of immunocompromised people; changes in food handling and consumption; and the emergence of new or antibiotic-resistant pathogens all contribute to increasing food safety risks (Unnevehr, 2003). Food safety requirements in export markets have a profound impact on the way that supply chains for agricultural and food products in developing countries operate. Food safety regulations and standards are increasingly influencing the ability of developing countries to access markets for agricultural and food products, particularly in industrialized countries (Henson, 2003a).

Food safety is of particular concern in developing countries not only because of the high prevalence of the food borne illness and other hazards associated with food, but also because of the considerable economic and social cost that, in turn, reflects prevailing levels of economic development. The majority of trade-limiting factors in developing countries relate to economics, poor infrastructure and lagging skills; food safety is still mainly the responsibility of the consumers. Improving food safety along western standards, however, may carry considerable costs and price food out of reach of the poor (Veen, 2005).

The center for disease Control and prevention has estimated that *Salmonella* infections were responsible for 1.4 million annual illnesses, resulting in nearly 600 deaths in 2003 in United States (CDC, 2003). CDC estimates that 1.4 million illnesses occur each year in the USA from the more than 2,000 strains of *Salmonella*, with infections associated with this pathogen resulting in more than 16,000 hospitalizations and nearly 600 deaths.

More severe cases of salmonellosis tend to occur in the very old, the very young, and the immunocompromised. The estimated annual costs in dollars in 1998 and 2003 of medical care and lost productivity due to food-borne *Salmonella* infections in the United States were \$2.3 and \$2.9 billion (Frenzen *et al.*, 1999, ERS-USDA, 2003).

A total of 150165 cases of human salmonellosis have been reported in 14 member states of the European Union in the year 2000 (European Commission, 2000b) and 192703 cases in 2004 (Forshell and Wierup, 2006). In the year 2000, 16 983 laboratory confirmed cases of salmonellosis were reported in the United Kingdom (UK Zoonoses Report, 2000). In the year 2001, China recorded that 17.9% of the total food poisoning was caused by *Salmonella* spp. (FAO/WHO, 2004). The trend in Salmonellosis in Australia has been increasing over the time, both in the number of cases recorded as well as in rate per population (Sumner *et al.*, 2004).

2.14. Control measures

Breaking the cycle of *Salmonella* from the feed animal to the consumer has proven to be very difficult. The United States and many European countries have evaluated several approaches to eradicate or at the very least reduce the incidence of salmonellosis (Marianne and Jeffrey, 2003).

The first line of defense against *Salmonella* in the food supply is control on the farm. Large scale and intensified farming practices that confine many animals or fowl in close quarters has significantly contributed to the increase in Salmonellae seen in the food supply. *Salmonella* inadvertently introduced into a herd or flock quickly spreads since the confined conditions expose the stock to contaminated feed and water through contact with infected animals and animal feces. Several methodologies are being evaluated to inhibit the spread of Salmonellae on the farm. One technique currently being evaluated is competitive exclusion, a method that uses defined bacterial flora to compete with Salmonellae for colonization of the ceca and other tissues (Schneitz and Hakkinen, 1998).

Vaccination is another alternative being assessed in several eradication programs. Results from the competitive exclusion and vaccine trials indicate that Salmonellae control on the farm may be feasible. However, complete eradication may prove difficult. Therefore, control of bacterial contamination during slaughter and processing is also necessary to decrease *Salmonella* present in the food supply (Schneitz and Hakkinen, 1998).

Furthermore, the HACCP system was designed to identify potential sources of contamination during slaughter and processing. This program consists of identification and assessment of

hazards associated with all stages of food processing, determination of critical points at which identifiable hazards can be controlled, and establishment of procedures to monitor the identified control points to determine whether or not a hazard does occur. HACCP is a systematic approach to food safety where a procedure is set up to both establish food safety and monitor and quickly correct problems (Guthrie, 1992).

A pathogen reduction and HACCP plan for *Salmonellae* have recently been implemented in several large meat and poultry plants in the United States (USDA, 1998.). Preliminary results indicate that *Salmonella* prevalence in broiler carcasses dropped from 20% before the program to 10.4% after implementation. For swine carcasses, prevalence dropped from 8.7 to 5.5%. Although these are preliminary data, they suggest that HACCP programs can reduce *Salmonella* in the food supply.

Education of the consumer is also critical for the control of salmonellosis. Making the consumer aware that *Salmonella* is present in foods such as fresh poultry and eggs is critical in the control of disease. More important, consumers must be made aware of potential cross-contamination from cooking utensils such as cutting boards and knives to foods that will not be cooked. Finally, simple hygiene such as hand washing before food preparation will greatly reduce the number of cases of *Salmonella* food poisoning seen each year (Marianne and Jeffrey, 2003).

Continuous surveillance and careful reporting of *Salmonella* isolates also contributes to control of disease. This surveillance improves awareness of new serotypes, common sources, antibiotic resistance, and carrier state (Marianne and Jeffrey, 2003).

3. MATERIALS AND METHODS

3.1. Study site

The study was conducted in Debre Zeit. Debre Zeit is a town of Ethiopia, lying south east of Addis Ababa. It is also known by the Oromo name, Bishoftu, which was its name until 1955. The town is located in the Misraq Shewa Zone of the Oromia Region, and has a latitude and longitude of 8°45'N 38°59'E with an elevation of 1920 meters. It is the administrative center of Adaa Chukala woreda (http://en.wikipedia.org/wiki/Debre_Zeit).

Based on figures from the Central Statistical Agency in 2005, Debre Zeit has an estimated total population of 131,159, of whom 64,642 are men and 66,517 women. The three largest ethnic groups reported for this town were the Amhara (42.86%), the Oromo (39.4%), and the Gurage (8.3%); all other ethnic groups made up 9.44% of the population. The 1994 census reported Debre Zeit had a total population of 73,372 of whom 35,058 were men and 38,314 were women ([http://en.wikipedia.org/wiki/Central_Statistical_Agency\(Ethiopia\)](http://en.wikipedia.org/wiki/Central_Statistical_Agency(Ethiopia))).

Cottage cheese sold in Debre Zeit open market is brought from the town and *kebeles* near the town. Cheese is sold in this market only on market days; Sunday, Thursday and Saturday. Most sellers bring the cheese usually with plastic containers covered by leaf. While purchasing, Testing, smelling and touching cheese with bare hand is common and normal practice in this market.

In Adaa Dairy Processing Association and Genesis farms, Just after production cheese is weighed in 1 and 0.5 kg and packed with plastic, then stored in refrigerator at +4 °C until it is sold. The cheese in these plants is not usually stored for more than 6 hours (usually sold soon).

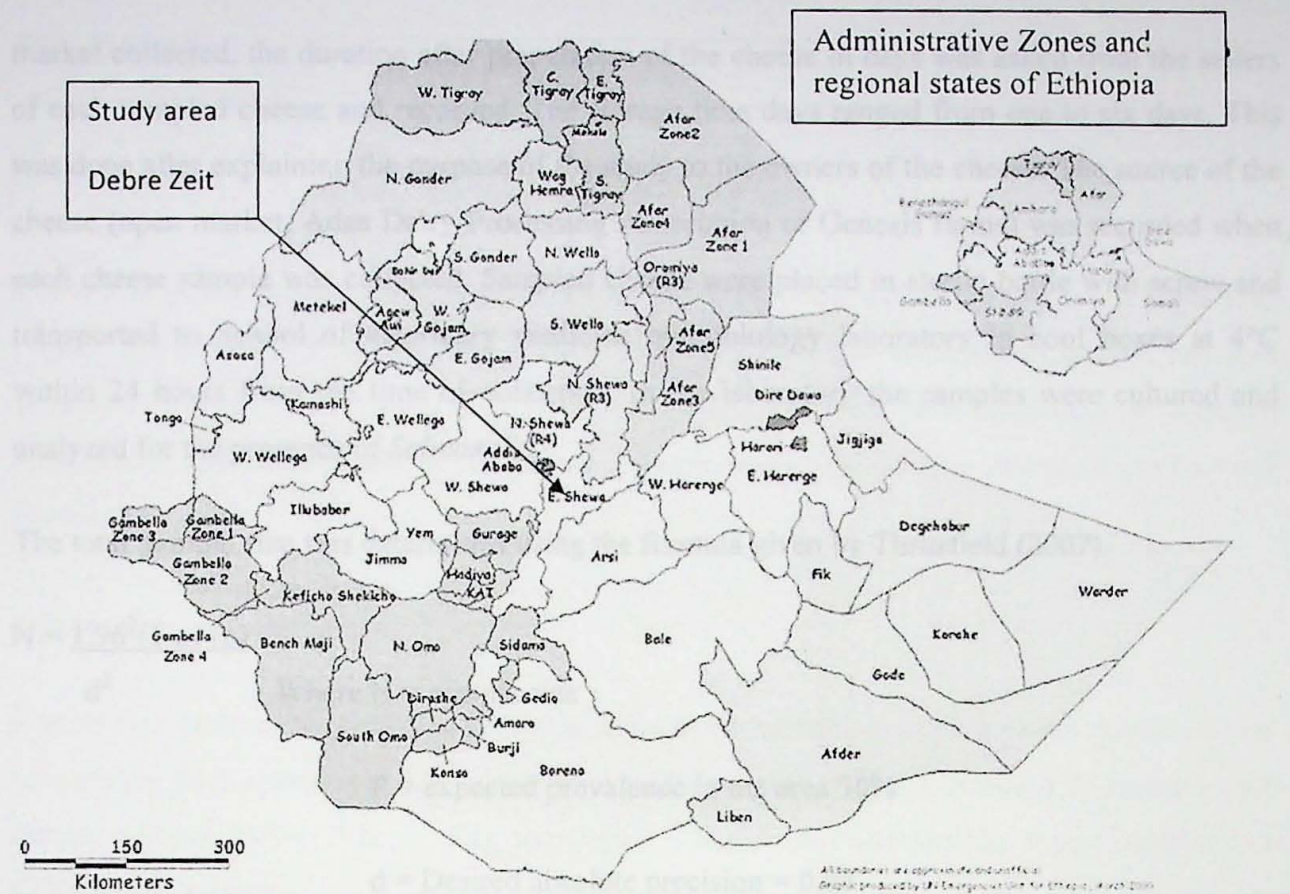


Figure 1 map of the study area

3.2. Study design

A cross sectional type of study was conducted from September 2010 to June 2011 to determine the prevalence of *Salmonella* in cottage and pasteurized cheese.

3.3. Sampling and study population

The study was conducted on cottage cheese from Debre Zeit open market and pasteurized cheese from Adaa Dairy Processing Association and Genesis farms.

Twenty five gram (Global Salm-Surv, 2003) of cheese samples were randomly selected and directly collected from cheese vendors at Debre Zeit open markets. Where as, the samples from Ada'a Dairy Processing Association and Genesis farms were taken from pasteurized and plastic packed cheeses which were supposed to be pathogen free. When samples from Debre Zeit open

market collected, the duration after preparation of the cheese in days was asked from the sellers of each sampled cheese and recorded. The storage time days ranged from one to six days. This was done after explaining the purpose of the study to the owners of the cheese. The source of the cheese (open market, Adaa Dairy Processing Association or Genesis farms) was recorded when each cheese sample was collected. Sampled cheese were placed in sterile bottle with screw and transported to School of veterinary medicine microbiology laboratory in cool boxes at 4°C within 24 hours from the time of collection. In the laboratory the samples were cultured and analyzed for the presence of *Salmonella*.

The total Sample size was determined using the formula given by Thrusfield (2007).

$$N = \frac{1.96^2(1-p)(p)}{d^2}$$

Where N = sample size

P = expected prevalence in the area 50%

d = Desired absolute precision = 0.05

Accordingly the sample size was 384. Out of the 384 cheese samples half 192 were cheese from unpasteurized source; Debre Zeit open market and 192 were cheese from pasteurized milk of which 96 were from Adaa Dairy Processing Association and 96 from Genesis farms. This sampling proportion was followed based on the daily follow up done and which showed almost comparable amount of cheese production between Adaa dairy processing Association and Genesis farms.

3.4. Methods

3.4.1. Isolation

The isolation and identification of *Salmonella* was carried out according to Global Salm-Surv (2003) and ISO 6579 (2002) with minor modifications. The isolation procedure has five successive stages; Non-selective pre-enrichment, Selective enrichment, Spreading on selective agar plates, Sub cultivation of *Salmonella* suspect colonies on non selective media, and Biochemical tests.

Non-selective pre-enrichment

Twenty five gram of the collected cheese samples were put with a sterile wooden spatula into an Erlenmeyer flask containing 225 ml buffered peptone water (AES laboratoire) to obtain 1 part sample + 9 part buffer. After mixing thoroughly using the wooden spatula, it was incubated at 37°C for 16-20 hours.

Selective enrichment

0.1 ml of the pre-enrichment was taken with sterile a pipette and transferred into 10 ml of Rappaport -Vassiliadis (RV) broth and the tubes were incubated at $41.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 18- 24 hours. This was done to increase the chance of the recovery of the target organism.

Spreading on selective agar plates

Loop full from the inoculated and incubated RV broth was taken and speared on two selective media Xylose Lysine Desoxycholate (XLD) (AES laboratoire) and Difco™ *Salmonella Shigella* (SS) agar then the plates were incubated at 37°C for 18-24 hours.

Sub cultivation of Salmonella suspect colonies

After reading the XLD and SS agar plates, *Salmonella* suspected colonies on XLD and SS agar (five presumptive *Salmonella* colonies) were selected and plated onto non-selective media, nutrient agar (Oxoid Ltd., Basingstoke Hampshire, England). The *Salmonella* presumptive colonies inoculated on to nutrient agar were incubated for 24 hours at 37°C. These plates were used for biochemical confirmation of *Salmonella* and serotyping.

Colonies on XLD were taken as typical for *Salmonella*, when they have slightly transparent zone of reddish color and a black centre and also when a pink-red zone was seen in the media surrounding the colonies. Typical *Salmonella* growth on XLD were Marked with a + in the result recording sheets.

Typical *Salmonella* colonies on an SS agar plate are Colorless, usually with black center. When there is Typical *Salmonella* growth on SS agar it will be marked with a + in the result recording sheets.

3.4.2. Biochemical tests

Biochemical tests were done to determine whether the colonies resembling *Salmonella* on XLD and SS agar are *Salmonella* or not. Five presumptive *Salmonella* colonies from XLD and SS agar were sub cultured on nutrient agar (non-selective media), for biochemical confirmation of *Salmonella* and serotyping. The biochemical tests that have been done were triple sugar fermentation test on Triple Sugar Iron (TSI) agar, Urease production test, lysine decarboxylase (LDC), Voges Proskauer VP and Indole production test (ISO 6579, 2002).

Triple sugar fermentation test

Triple sugar iron agar (Oxoid) dehydrated medium was used for this test and slants were prepared according to the manufacturer's instruction. After preparing the slants, the inoculation was done carefully by touching only the center of an isolated colony from the nutrient agar plated medium with a straight, cool, sterile needle. Then stabbing the medium in the butt of the tube, and then streaking back and forth along the surface of the slant. The slants were incubated at 36 °C for 18-24 hours.

Results were interpreted as glucose fermented when only the butt of the slants becomes acidic (yellow) and as lactose and/or sucrose fermented when the slant become acidic (yellow). When the PH of the medium is not changed (TSI tube remains red) it was indication for absence of fermentation of glucose. The presence of black precipitate indicated hydrogen sulfide production. Gas production is indicated by splits or cracks in the butt of the agar were taken as indication for gas production (BD Microbiology Systems, 1992; Koneman *et al.*, 1997).

Indole production test

Indole production test was done using SIM BBL™ medium. Tubes were prepared according to the manufacturer's instruction. The tubes were stabbed with fresh culture of suspected colonies from nutrient agar. The tubes were incubated at 37 °c for 18 to 24 hours. After incubation three drops of kovac's reagent was added and the results were read after 15 seconds. The result of the indole production test was interpreted positive when red color develop at the top of the agar surface and negative if color change is not observed (if it remain yellow which is the color of the kovacs reagent) (BD Microbiology Systems, 1997).

Urease production test

This test was done by streak back and forth over the entire slant surface using a heavy inoculum of growth from 24-hours pure culture. The butts of the tubes were not stabbed. Tubes were incubated with loosened caps at 36°C. Reactions were observed after 24 and 48 hours.

The production of urease was confirmed when an intense pink-red (red-violet) color on the slant or throughout the agar was observed and negative when there was no color change i.e. The agar medium remains pale yellow.

Voges–Proskaure test

Voges–Proskaure test has been conducted to detect acetoin production. A 5 ml of MR-VP (methyl red voges proskaure) broth in a tube was inoculated with the suspected organism and incubated at 36°C for 18–24 hrs. After incubation, 2.5 ml of the broth culture was transferred to a tube and 6 drops of alpha naphthol was added followed by 3 drops of KOH. Then it was waited for the development of color change. The test was considered positive when red color was

developed within 15 minutes. No color change indicates negative VP (Koneman *et al.*, 1997; Murray *et al.*, 2003).

Lysine decarboxylase (LDC) test

The lysine decarboxylase test was done according to the procedure indicated by WHO and CDC (2003). The slants were made with care so that the tubes will have deep butt as the decarboxylation reaction occurs only in anaerobic conditions.

Lysine iron agar (LIA) (BBL™) was used to undertake LDC test. After preparing, the slants were inoculated with the test isolate by stabbing base of tube butt and streaking the slant with a needle. Then the tube caps were loosened to ensure aerobic conditions and incubated at 35°C for 18 - 48 hours. Tubes were examined at 24 and 48 hours for growth and color changes in tube butt and slant and for blackening at the apex of slant. Positive lysine decarboxylase reaction was considered when purple (alkaline) butt and purple slant developed. The test was recorded as negative if the butt and the slant are yellow (acid) and purple (alkaline) respectively and positive hydrogen sulfide reaction when blackening of the medium at the apex of the slant was observed.

3.4.3. Culturing on chromogenic agar media

Those isolates which were positive for *Salmonella* by biochemical test and those which were inconclusive or doubtful were grown on CHROMagar™ *Salmonella* Plus medium which is a chromogenic medium for the isolation of *Salmonella* species including *S. Typhi* and lactose positive *Salmonella*. This medium meets the ISO 6579: 2002 ISO norm. *Salmonella* growth on this medium was confirmed based on the color of the colonies and those produced typical mauve colonies were taken as *Salmonella* species.

3.4.4. Serotyping of *Salmonella* isolates

The isolates which were positive by biochemical and on chromogenic medium have been sent to reference laboratory in India for serotyping.

3.5. Data analysis

Data were put in to Microsoft excel and the analysis of these data was done using version 15 of statistical package for social science (SPSS). Percentages were used to express the proportion. The difference in isolation rate of *Salmonella* between the two types of cheeses and storage time was analyzed using multivariate logistic Regression and chi square analysis. The results of this study are reported as significant when P-value is found to be less than 5 %.

Growth on SSA/ XLD	Number of sample	Typical <i>Salmonella</i> colonies	Total
Grown	220	20	220
Not grown	164	0	164
Total	384	20	384

4.2. Storage time of cottage cheese and *Salmonella* positivity

While collecting cottage cheese from Debra Zait open market storage time, the time from production of the cottage cheese to market was recorded by asking the vendor cheese sellers. The storage time has ranged from 1 day to 6 days. The samples were analyzed for the presence of *Salmonella* and Table 2 shows the number of *Salmonella* isolated with storage time of the cottage cheese. Accordingly, 4 *Salmonella* isolates were isolated from cottage cheese of storage time 1 day and there were two isolates from cheese of storage time 2 days but there were no isolation of *Salmonella* from cottage cheese of storage time of 3 days and above.

There was statistical significant ($P = 0.027$) difference in the isolation rate of *Salmonella* from cottage cheese with the storage time. The higher positivity for *Salmonella* was seen in cheese samples stored only for 1 day followed by cheese stored for two days.

4. RESULTS

4.1. Presence of growth on SS and/or XLD agar

After selective enrichment on RV broth, Streaking from the incubated RV broth on to SSA and XLD selective media was done. On plates of XLD and SS agar, growth of typical colonies for *Salmonella* and any growth were observed and recorded. Table 1 indicates the number of typical *Salmonella* colonies grown SSA/XLD and any bacteria that have grown on theses media.

Table 1 Typical *Salmonella* and non typical bacteria grown on SSA and/or XLD media

Growth on SSA/ XLD	Number of sample	Typical <i>Salmonella</i> colonies	Total
Grown	220	20	220
Not grown	164	0	164
Total	384	20	384

4.2. Storage time of cottage cheese and *Salmonella* positivity

While collecting cottage cheese from Debre Zeit open market storage time, the time from production of the cottage cheese to market was recorded by asking the vender cheese sellers. The storage time has ranged from 1day to 6 days. The samples were analyzed for the presence of *Salmonella* and Table 2 shows the number of *Salmonella* isolated with storage time of the cottage cheese. Accordingly, 4 *Salmonella* isolates were isolated from cottage cheese of storage time 1 day and there were two isolates from cheese of storage time 2 days but there were no isolation of *Salmonella* from cottage cheese of storage time of 3 days and above.

There was statistical significant ($P = 0.027$) difference in the isolation rate of *Salmonella* from cottage cheese with the storage time. The higher positivity for *Salmonella* was seen in cheese samples stored only for 1 day followed by cheese stored for two days.

Table 2 Storage time of the cottage cheese and *Salmonella* positivity

Storage time*	No. of samples	Positive for <i>Salmonella</i> n (%)	Odds ratio (95% confidence interval)	P value
1 day	66	4 (6.06)	1.03 (.017 - 6.30)	0.027
2 day	58	2 (3.45)	0.91 (0.15 - 5.50)	
3 day	38	0	0.59 (0.10 - 3.61)	
4 day	25	0	-	
5 day	3	0	-	
6 day	2	0	-	

★ The time from production to market

4.3. *Salmonella* isolates in cheese type, source and storage time

Of the 384 cheese samples which were subjected to bacteriologic analysis, *Salmonella* was isolated from 6 (1.6%) of them. With regard to source of the cheese samples, all the 6 *Salmonella* were isolated from cheese sample taken from Debre Zeit open market and there were no isolates from cheese samples from Adaa Dairy Processing Association and Genesis farms. The results showed that there is significantly ($P = 0.015$) higher *Salmonella* in cheese from Debre Zeit open market than cheese from Adaa Dairy Processing Association and Genesis farms.

Table 3 *Salmonella* isolates in cheese type, source and storage time

Variables		No. examined	Positive for <i>Salmonella</i> (%)	Chi square	F value
Heat treatment	Pasteurized	192	0	8.41	0.004
	Non pasteurized	192	6 (3.13)		
Sample source	Adaa Dairy processing Association	96	0	8.41	0.015
	Genesis farms	96	0		
	Debre Zeit open market	192	6 (3.15)		
Storage time	1 day	66	4 (6.06)	14.23	0.027
	2 days	58	2 (3.45)		
	3 days	38	0		
	4days	25	0		
	5 days	3	0		
	6 days	2	0		
Growth on SSA/XLD	Growth	220	6 (2.72)	6.76	0.009
	No growth	164	0		

5. DISCUSSION

Cheese could be made from pasteurized or unpasteurized milk; and the major purpose of milk pasteurization is to eliminate potential pathogens that may be found in raw milk. Heat treatment of milk has been shown to affect the microbiological and chemical composition of cheese (Xanthopoulos *et al.*, 2000). Cottage cheese or “ayib” is an Ethiopian traditional dairy product made from sour milk after the removal of the fat by churning and cooking the curd to a temperature of 40 – 70 °C. “Ayib comprises 79% water, 14.7% protein, 1.8% fat, 0.9% ash and 3.1% soluble milk constituents (O’Mahony, 1988).

In this study, the overall *Salmonella* prevalence in study samples (cottage and cheese made from pasteurized milk) was low 6 out of 384 (1.6%) This could be partly because half of the cheese samples were made from pasteurized milk and in that case the pasteurization temperature is expected to kill the common pathogens including the target pathogen of this study. The heating temperature used for cottage cheese making around the study area could have contributed for the relatively low *Salmonella* prevalence. But it does not mean that this level of *Salmonella* prevalence lacks attention. This is due to the fact that low dose of *Salmonella* potentially required to result in infection leading to a variety of clinical conditions and septicemia and even deaths. It suggests that raw milk cheeses can pose a public health hazard, especially for people with compromised immune systems. Cottage cheese, although fermented, cannot be totally excluded from being source of infection for humans. Furthermore the large number of HIV and TB patients in our country increased the number of immunocompromised individuals. It is therefore; clear that *Salmonella* prevalence 3.22% in cottage cheese in the present study can pose public health risk to the consumer. According to the 2007 Single Point Estimate, there were an estimated 1,116,216 people living with HIV in 2009 (FDRE, 2010). Ethiopia ranks seventh among the world’s 22 high-burden tuberculosis (TB) countries. According to the World Health Organization’s (WHO’s) Global TB Report 2009, the country had an estimated 314,267 TB cases in 2007 (WHO, 2009).

Endrias and Poppe (2009) have reported a comparable but higher prevalence, (2.1%) of *Salmonella*. The overall prevalence of *Salmonella* in this study was 1.6% in cheese. This may be

due to the difference in cheese type between the two studies and the differences in cheese making temperature in the areas the cheeses were obtained. The present study was done on cottage and cheese from pasteurized milk. In this case, the cheese from pasteurized milk accounted for the lower prevalence of the target pathogen. There are other researchers who have reported similar results (Abd El-Atty, 2007).

In a study made in Brazil, with the objective to evaluate the occurrence of pathogens and indicators of hygienic-sanitary cheese curd produced by hand, a very high prevalence has been reported, in which 34.9% of cheese samples were found to be positive for *Salmonella* (Borges *et al.*, 2003). The big difference in the prevalence of *Salmonella* in cheese could be due to difference in the production condition (cheese making temperature and personal hygiene status of handlers) and initial microbial load of the milk used for cheese making. Yagoub and his colleagues (2006) have reported comparable but still higher *Salmonella* prevalence from 6% of the white cheese samples collected from retailer shops and restaurants in Khartoum and Omdunna cities during the period from February to November 2001.

In another study conducted in Turkey to determine the prevalence of *Salmonella* spp. and *Listeria monocytogenes* in different cheese types produced in Turkey, 1.9% of white cheeses were positive for *Salmonella*. The findings of Tolga and his colleagues (2010) is almost the same with the observations of present study.

Statistically significant difference ($P = .004$) have been observed in prevalence of *Salmonella* between cottage cheese 6 (3.13%) and cheese made from pasteurized milk 0%. There is no work done in other part of the country on *Salmonella* from cheese from pasteurized milk. However, from Canada, there is a documented food poisoning outbreak caused by *S. Muenster* occurred in 1982 implicated (cheddar cheese made from unpasteurized milk) food as source of infection (Wood *et al.*, 1982). Some pathogens are inactivated during the cheese making process (this depends on the temperature and pH during production and ripening), but many microbes can survive. Clearly the process in itself cannot be relied upon to eliminate them; adequate pasteurization, is therefore, the logical first stage in the manufacture of cheese (ECD, 1992).

In this investigation, an attempt was also made to see if there is difference in the isolation rate of the target pathogen among cottage cheese stored for different time length. Out of the 6

Salmonella isolates, 4 of them were from cottage cheese stored for only 1 day and two of the isolates were from cheese of two days of storage and the difference in the isolation rate of *Salmonella* with the storage time was statistically significant ($P = 0.027$). Higher recovery was seen in cottage cheese stored for 1 day (6%) followed by cheese stored for two days (3.5%) and no *Salmonella* was recovered from cottage cheese sample stored for more than two days. this difference in the rate of recovery of the target pathogen could be due to the increase in acidity of the cottage cheese as storage time increase which is usually stored at ambient temperature until it is brought to market. There are very few researches conducted on effect of storage time on the fate and survival of *Salmonella* in cottage cheese. Due to this reason, most of the discussion made here is based on the results obtained from studies on other Ethiopia foods.

Mohammed *et al.* (1996) studied survival and growth of *Salmonella* during the making of 'ayib' a traditional Ethiopian cottage cheese made from sour milk after the fat is removed by churning. According to their study, *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella infantis* were able to grow when added to raw cheese milk, but none was able to survive the heating process at the prevailing low pH. When the *Salmonella* test strains were added after heating, they were able to survive for over 24 hours. They disappeared only after three days. The finding of these investigators is highly in agreement with the results of the present study in which *Salmonella* was isolated from cottage cheese of maximum storage time of two days. Similarly Girma *et al.* (1989) have studied survival of *Salmonella* in teff when it is fermented and found the growth of *Salmonella typhimurium*; and other three pathogens to be inhibited when the pH of fermenting teff reached 5.0 - 5.5.

In another the study, Dessie *et al.* (1996) compared the fate of two *Salmonella* test strains in fermenting *siljo* and a control gruel which was not made to ferment. They could not grow the test strains in fermenting *siljo*. *S. enteritidis* and *S. typhimurium* were completely eliminated at 48 and 72 hours. Ephraim and Ashenafi (2005) also evaluated the fate of *Salmonella typhimurium* DT 104 during the fermentation of *siljo* at ambient and cold temperatures. When they inoculated *Salmonella typhimurium* DT 104 fermenting gruel at low initial levels (2.8 log cfu/ml), then count decreased steadily and the test strain was not detected by enrichment on day 5 but it the in control, the test strain increased by about 3 log units on day 7. 'Siljo' is a fermented product made from safflower (*Carthamus tinctorius*) extract and faba bean (*Vicia faba*) flour. It is a

popular condiment during the long fasting period before Easter (Hesseltine, 1983). Inoculation of *Salmonella typhimurium* in fermenting *awaze* (a traditional fermented condiment and is consumed with other items) resulted in a fast elimination of the pathogen (Idris *et al.*, 2001).

This study has indicated the less survival of *Salmonella* species in cheese as the storage time of cottage cheese increase. This could be due to the increase in the acidity of cottage cheese when stored usually at room temperature at household.

6. CONCLUSION AND RECOMMENDATIONS

After successful completion of the present study, *Salmonella* has been isolated from cottage cheese (3.13%) and the effect of storage time on the isolation rate of *Salmonella* has been known. The survival and growth of *Salmonella* decreased as the storage time of cottage cheese is increasing this is possibly due to increase in the acidity of the cheese. Even though the prevalence of *Salmonella* in cottage cheese found in the present study is not higher, due to the ready to eat nature of the food it can pose great public health concerns even with low incidence of contamination. Cheese made from pasteurized milk is found to be negative for *Salmonella* in this study. This finding indicated the effectiveness of the pasteurizing temperature in the plants from which the samples were collected.

Based on the conclusions made above the following recommendations are forwarded

- ❖ Due attention need to be given for the safety and quality of foods of animal origin in general and ready to eat foods like cheese in particular
- ❖ Stakeholders in the dairy sector should work in organizing small scale dairy farmers to processes their milk product by forming associations
- ❖ To minimize the public health risk that arise from consumption of raw milk products, education and awareness creation should be done with the small scale farmers to increase their understanding about the importance of using appropriate temperature in making milk products
- ❖ Consumers should use only pasteurized products when it is feasible
- ❖ further studies need to be conducted on other pathogens in cheese and other food types

7. REFERENCES

- Abd El-Atty, N. and Meshref, A. (2007): Prevalence of *Salmonella* and *E. coli* O157 in some foods. BS. Vet. Med. J., 5th scientific conference. Pp. 73-78.
- Acha, P. and Szyfres, B. (2001): Zoonoses and communicable Disease common to man and animals. 3rd Edition, Washington DC Pan American Health Organization, Pp. 233-246.
- Alakomi, H. and Saarela, M. (2009): *Salmonella* importance and current status of detection and surveillance methods. Quality Assurance and Safety of Crops and Foods, **1**: 142–152.
- Alphons, A., van, A., Jaap, E. and van, D. (2005): Mini review: Distribution of “classic” virulence factors among *Salmonella* spp. *FEMS. Immun. Med. Microbiol.*, **44**: 251-259.
- Altekruse, S., Timbo, B. and Mowbray, J. (1998): Cheese-associated outbreaks of human illness in the United States, 1973-1992: Sanitary manufacturing practices protect consumers. *J. Food Protect.*, **61**: 1405- 1407.
- Anon (1996a): *Salmonella* gold-coast. *Commun. Dis. Report Wk.*, **6**: 443.
- Anon (1996b): *Salmonella* gold coast and Farm Cheese. Food hazard warning. Department of Health, London UK 18 December 1996.
- Anon, (1997): *Salmonella* gold-coast and cheddar cheese: update. *Commun. Dis. Report Wk.*, **7**: 93-96.
- Arun, K. (2008): Food borne Microbial Pathogens, Mechanisms and Pathogenesis, Purdue University West Lafayette USA. Pp. 9, 27, 221.
- Aseffa, A., Gedlu, E. and Asmelash, T. (1994): Antibiotic resistance of prevalent *Salmonella* and *Shigella* strains in northwest Ethiopia. *East Afr. Med. J.*, **74**: 708-13.
- Ashenafi, M. (1989): Proteolytic, lipolytic and fermentative properties of yeasts isolate from ayib, a traditional Ethiopian cottage cheese. SINET: *Eth. J. Sci.*, **12**: 131-139.

- Asrat, D. (2008): *Shigella* and *Salmonella* serogroups and their antibiotic susceptibility patterns in Ethiopia. *Eatern. Mediterr. Hlth. J.*, **14**: 760-767.
- Awole, M., Gebre-Selassie, S., Kassa, T. and Kibru, G. (2002): Isolation of potential bacterial pathogens from the stool of HIV-infected and HIV-non-infected patients and their antimicrobial susceptibility patterns in Jimma Hospital, south west Ethiopia. *Eth. Med. J.*, **40**: 353-64.
- BD Microbiology Systems (1992): Quality Control and Product information Manual for Tabled Media. BD Microbiology Systems Cockeysville, MD.
- Beatty, E., Shevick, G., Shupe-Ricksecker, K., Bannister, E., Tulu, A. and Lancaster, K. (2009): Large *Salmonella* Enteritidis outbreak with prolonged transmission attributed to an infected food handler, Texas, 2002. *Epidem. Infect.*, **137**: 417-427.
- Bell, C. and Kyriakides, A. (2002): *Salmonella*: a practical approach to the organism and its control in foods. Publisher: Oxford; Malden, Mass. Blackwell Science Ltd. Pp. 70- 71, 202, 221, 269.
- Besser, T., Goldoft, M. and Pritchett, L. (2000): Multiresistant *Salmonella* Typhimurium DT104 infections of humans and domestic animals in the Pacific Northwest of the United States. *Epidem. Infec.*, **124**: 193–200.
- Beyene, G. Asrat, D., Mengistu, Y., Aseffa, A. and Wain, J. (2008): Typhoid fever in Ethiopia. *J. Infect. Dev. Ctries.*, **1**: 448-453.
- Beyene, G., Satheesh, N., Daniel, A., Yohannes, M., Howard, E. and John, W. (2011): Multidrug resistant *Salmonella* concord is a major cause of salmonellosis in children in Ethiopia. *J. Infect. Dev., Ctries.*, **5**: 23-33.
- Blaser, J. and Newman, S., (1982): A review of human salmonellosis. I Infective dose. *Rev. Infect. Dis.*, **4**: 1096-1106.

- Borges, D., Terezinha, F., Renata, N., Celle, M., Erika, A., Evan, A. and Teixeira, D. (2003): Microorganisms pathogenic indicators and cheese curd produced in the state of Ceará, Brazil, Bulletin of the Research Center of Food Processing, **21**(1). ISSN: 19839774.
- Brenner, F., Villar, R., Angulo, F., Tauxe, R. and Swaminathan, B. (2000): *Salmonella* nomenclature. *J. Clin. Microbiol.*, **38**: 2465-2467.
- CDC, Centers for Disease Control and Prevention (2002): Outbreak of multi-drug resistant *Salmonella* Newport-United States, January–April 2002. MMWR 51:545.
- CDC, Centers for Disease Control and Prevention (2003): Multistate outbreak of *Salmonella* serotype Typhimurium infections associated with drinking unpasteurized milk-Illinois, Indiana, Ohio, and Tennessee, 2002–2003. MMWR 52: 613.
- CDC, Centers for Disease Control and Prevention (2008): *Salmonella* Surveillance: Annual Summary, 2006 US Department of Health and Human Services. Atlanta Georgia.
- CDC, Centers for disease control and prevention (2009): surveillance for food disease outbreak, United States, 2006. Morbidity and Mortality weekly Report, **58**: 609-615.
- CDC, Centers for Diseases Control and Prevention (2007a): Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter-United States, 2006-2007. MMWR., **56** :521-524.
- CDC, Centers for Diseases Control and Prevention (2007b): *Salmonella* serotype Enteritidis infections among workers producing poultry vaccine-Maine, November-December 2006. Morbidity and Mortality Weekly Report, **56**: 877-879.
- CDC. Centers for Diseases Control and Prevention (2008): Multistate outbreak of human *Salmonella* infections associated with exposure to turtles-United States, 2007-2008. Morbidity and Mortality Weekly Report 2008a; **57**: 69-72.
- Cheesbrough, M. (1985): Medical Laboratory Manual for Tropical Countries. Vol.2. Microbiology Pp. 400-480.

- CIDRAP (2006): Centre for Infectious Disease Research and Policy, Academic Health Centre, University of Minnesota. <http://www.cidrap.umn.edu/cidrap/contents/fs/food-disease/causes/salmonview.html> (Accessed on 03/05/2007).
- Cody, S., Abbott, S. and Marfii, A. (1999): Two outbreaks of multidrugresistant *Salmonella* serotype Typhimurium DT104 infections linked to rawmilk cheese in Northern California. *J. Amer. Med. Ass.*, **281**: 1805- 1810.
- D'Aoust, J. (1989): *Salmonella* Food borne Bacterial Pathogens (ed. M.P. Doyle). Marcel Dekker, Inc., New York, USA. Pp. 327-445.
- D'Aoust, J., Aris, B. and Thisdele, P. (1975): *Salmonella* eastbourne outbreak associated with chocolate. *J. Instit. Canad. Sci. Techn. Aliment.*, **8**: 181-184.
- D'Aoust, J., Warburton, D. and Sewell, A. (1985): *Salmonella* typhimurium phage type 10 from cheddar cheese implicated in a major Canadian food borne outbreak. *J. Food Protect.*, **48**: 1062-1066.
- D'Aoust, Y., Emmons, B., McKellar, R. (1987): Thermal inactivation of *Salmonella* species in fluid milk. *J. Food Protect.*, **50**: 494-501.
- De Valk, H., Delarocque-Astagneau, E. and Colomb, G. (2000): A communitywide outbreak of *Salmonella enterica* serotype Typhimurium infection associated with eating a raw milk soft cheese in France. *Epid. Infect.*, **124**:1-7.
- Deb, M. and Kapoor, L. (2005): *Salmonella* nomenclature seen in the literature. *Ind. J. Med. Microbiol.*, **23**: 204-205.
- Dessie, G., Kebede, A. and Mogessie, A. (1996): Fate of *Salmonella enteritidis* and *Salmonella typhimurium* during the fermentation of 'siljo', a traditional Ethiopian fermented product. *East Afr. Med. J.*, **73**: 432-434.

- ECD, European Council directive (1992): Laying down the health rules for the production and placing on the market of milk and milk-based products, as amended by council directives 92/118/EEC and 94/71EC. Official Journal 1994; No L268:1. (14 September.)
- Endrias, Z., and Poppe, C. (2009): Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa, Ethiopia *Trop. Anim. Hlth. Prod.*, **41**: 241–249.
- Ephraim, E. and Ashenafi, M. (2005): Fate of *Salmonella typhimurium* DT 104 during the fermentation of ‘Siljo’, a traditional Ethiopian fermented legume condiment, and during product storage at ambient and refrigeration temperatures. *World J. Microbiol. Biotechnol.*, **21**: 1259–1265.
- ERS-USDA (2003): Economic Research Service, United States Department of Agriculture. <http://www.ers.usda.gov/Briefing/FoodborneDisease/Salmonella.htm>. Accessed on August 8, 2007
- European Commission (2000a): *Salmonella*. In: Opinion of the scientific committee on veterinary measures relating to public health on food-borne zoonoses. Health and Consumer Protection Directorate General, Directorate B- Scientific Health Opinions, Unit B3-Management of Scientific Committees II, 12th April 2000. Pp. 24-27.
- European Commission (2000b): Trends and sources of zoonotic agents in animals, feeding stuffs, food and man in the European Union and Norway in 2000. Working document, Directorate D-Food Safety: Production and distribution Chain.
- European Commission (2003): Health and Consumer Protection Directorate-general Opinion of The Scientific Committee on Veterinary Measures relating to Public Health on *Salmonella* in Foodstuffs http://ec.europa.eu/food/fs/sc/scv/out66_en.pdf Accessed on May 15, 2011.
- FAO/WHO (2004): FAO/WHO Regional conference on food safety for Asia and Pacific. The national surveillance system for food-borne disease in China, Seremban, Malaysia, 24-27 May.

- FDA, Food and Drug Administration (2001): FDA survey of imported fresh produce. US Food and Drug Administration, Center for Food Safety and Applied Nutrition. www.cfsan.fda.gov.
- FDRE, Federal Democratic Republic of Ethiopia (2010): Report on progress towards implementation of the UN Declaration of Commitment on HIV/AIDS 2010 Federal HIV/AIDS Prevention and Control Office, Addis Ababa, Ethiopia.
- Fluit, A. C. (2005): Mini review: Towards more virulent and antibiotic-resistant *Salmonella*? *FEMS Immun. Med. Microbiol.*, **43**: 1-11. 69.
- Fontaine, R., Cohen, M. and Martin, W. (1980): Epidemic salmonellosis from Cheddar cheese: surveillance and prevention. *Amer. J. Epid.*, **111**: 247-253.
- Forshell, P. and Wierup, M. (2006): *Salmonella* contamination: A significant challenge to the global marketing of animal food products. *Scientific and Technical Review of the Office International des Epizooties* **25**: 541-554.
- Freitas, C., Penha, R., Barrow P. and Berchieri, A. (2010): Sources of Human Non-Typhoid Salmonellosis: A Review. *Braz. J. Poult. Sci.*, **12**: 01-11.
- Frenzen, D., Lynn, T., Buzby, C., Breuer, T., Roberts, T., Voetsch, D., Reddy, S. and the Food Net working group (1999): *Salmonella* cost estimate updated using Food Net data. *Food Rev.*, **22**: 10-15.
- FSM, Food safety magazine (2005): Testing *Salmonella* and *Campylobacter*: A Methods Update <http://www.foodsafetymagazine.com/article.asp?id=1183&sub=sub1> Accessed on May 15, 2011.
- Gebre-Yohannes, A. (1985): *Salmonella* from Ethiopia: prevalent species and their susceptibility to drugs. *Eth. Med. J.*, **23**: 97-102.
- Gedebou, M. and Tassew, A. (1981): Antimicrobial resistance and R factor of *Salmonella* isolates from Addis Ababa. *Eth. Med. J.*, **19**: 77-85.

- Girma, M., Berhanu, G. and Lakew, B. (1989): The effect of fermentation on the growth and survival of *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* in fermenting teff (*Eragrostis tef*). *MIRCEN. J. Appl. Microbiol. Biotechnol.*, **5**: 61-66.
- Global Salm-Surv (2003): A global *Salmonella* surveillance and laboratory support project of the World Health Organization 4th Edition. Laboratory Protocols Level 1 Training Course Kits for Identification of Enterobacteriaceae Using API.
- Gonfa, A., Foster, A. and Holzapfel, H. (2001): Field survey and literature review on traditional fermented milk products of Ethiopia. *Int. J. Food Microbiol.*, **68**: 173-186.
- Greenwood, M. and Hooper, W. (1983): Chocolate bars contaminated with *Salmonella* Napoli: an infectivity study. *Brit. Med. J.*, **286**: 1394.
- Gupta, A., Fontana, J. and Crowe, C. (2003): Emergence of multi-drug resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J. Infect. Dis.*, **188**: 1707-1716.
- Guthrie, R. (1992): *Salmonella*. Boca Raton, FL: CRC Press, Inc, USA.
- Hassan, L., Mohammed, H. and McDonough, P. (2000): A cross-sectional study on the prevalence of *Listeria monocytogenes* and *Salmonella* in New York dairy herds. *J. Dairy Sci.*, **83**: 2441-2447.
- Hendriksen, S., Mikoleit, M., Kornschöber, C., Ricket, R., Duyne, S. van Kjelso, C., Hasman, H., Cormican, M., Mevius, D., Threlfall, J., Valinsky, L., Angulo, J. and Aarestrup, M. (2009): Emergence of Multidrug-Resistant *Salmonella* Concord Infections in Europe and the United States in Children Adopted from Ethiopia, 2003-2007. *Pediat. Infect. Dis. J.*, **28**: 814-818.
- Hensel, M. (2004): Review; Evolution of pathogenicity islands of *Salmonella enterica*. *Int. J. Med. Microbiol.*, **294**: 95-102.

- Henson, S. (2003a): Food safety issues in international trade. In: Food safety in food security and food trade. 2020 vision for food, agriculture and the environment. Focus 10, brief 5 of 17. Pp. 15-20.
- Henson, S. (2003b): The Economics of food safety in Developing Countries. ESA Working Paper No. 03-19, December 2003 (www.fao.org/es/esa Accessed on 08/08/2007).
- Hesseltine, W. (1983): The future of fermented foods. *Nutr. Rev.*, **41**: 293-301.
- Hilal, C., Hamparsun, H., Enver, B. and Beyza, U. (2007): Prevalence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese. *Food Cont.*, **18**: 576-579.
- Hill, G. and Lillicrap, M. (2003): Arthritis associated with enteric infection. *Best Pract. Res. Clin. Rheumat.*, **17**: 219-239.
- [http://en.wikipedia.org/wiki/Central_Statistical_Agency_\(Ethiopia\)](http://en.wikipedia.org/wiki/Central_Statistical_Agency_(Ethiopia)). Accessed on December 1, 2010
- http://en.wikipedia.org/wiki/Debre_Zeyit. Accessed on December 1, 2010
- http://www.about-salmonella.com/salmonella_symptoms_risks. Accessed on June 1, 2011
- http://www.medpedia.com/news_analysis/148-Marlerblog/entries/16057-Salmonella-Montevideo-Outbreak-in-the-Offing. Accessed on May 15, 2011
- Idris, A., Tetemke M. and Ashenafi, M. (2001): Some microbiological and biochemical studies on the fermentation of "Awaze" and "Datta", traditional Ethiopian condiments. *Int. J. Food Sci. Nutr.*, **52**: 5-14.
- Inman, R., Johnston, M., Hodge, M., Falk, J. and Helewa, A. (1998): Postdysenteric Reactive Arthritis, A Clinical and Immunogenic Study following an Outbreak of Salmonellosis. *Arthr. Rheumat.*, **31**: 1377-1383.

- ISO 6579 (2002): Microbiology - General Guidance on Methods for the Detection of *Salmonella*, International Organization for Standardization 4th Edition, Geneva, Switzerland.
- James, M. and Detroit, M. (2000): Modern Food Microbiology 6th edition Aspen Publishers, Inc Gaithersburg, Maryland.
- Kaufman, A., Baron, A. and Kopp, E. (1966): Some effects of instructions on human operant behavior. *Psychon. Monogr. Supplem.*, **1**: 243-250.
- Koneman, E., Allan, S. and Janda, W. (1997): Color Atlas and Textbook of Diagnostic Microbiology, 5th Edition. J.B. Lippincott, Philadelphia.
- Le Minor, L. and Popoff, Y. (1987): Designation of *Salmonella enterica* sp. nov., nom. rev., as the type and only species of the genus *Salmonella*. *Int. J. Syst. Bacteriol.*, **37**: 465-8.
- Lehmacher, A., Bockemuhl, J. and Aleksic, S. (1995): Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika powdered potato chips. *Epid. Infect.*, **115**: 501-511.
- Lepage, P., Bogaerts, J., Nsengumuremyi, F., Hitimana, G., Van Goethem, C., Vandepitte, J. and Butzler, P. (1984): Severe multiresistant *Salmonella* typhimurium systemic infections in Central Africa--clinical features and treatment in a paediatric department. *J. Antimicrob. Chemother.*, **14** Suppl B: 153-159.
- Lindsay, J. (1997): Chronic sequelae of foodborne disease. *Emer. Infect. Dis.*, **3**: 443-52.
- Mache, A. (2002): *Salmonella* serogroup and their antibiotic resistance patterns isolated from diarrhoeal stools of paediatric out patients in Jimma Hospital and Jimma Health Center, South West Ethiopia. *Eth. J. Hlth Sci.*, **37**: 37-45.
- Maguire, H., Cowden, J. and Jacob, M. (1992): An outbreak of *Salmonella* dublin infection in England and Wales associated with a soft unpasteurized cows' milk cheese. *Epid. Infect.*, **109**: 389-396.

- Maguire, H., Cowden, J. and Jacob, M. (1992): An outbreak of *Salmonella* dublin infection in England and Wales associated with a soft unpasteurized cows' milk cheese. *Epidemiol. Infect.*, **109**: 389-396.
- Marianne, D. and Jeffrey, W. (2003): International Hand book of foodborne parhogens Marcel Dekker, INC. New York, Basel.
- Matheson, N., Kingsley, A., Sturgess, K., Aliyu, H., Wain, J., Dougan, G. and Cooke, J. (2010):
- Mayo Clinic (2007): *Salmonella*. Retrieved from Mayo Clinic Web site <http://www.mayoclinic.com/health/salmonella/DS00926> on August 29, 2007
- Mayo Clinic (2011): *Salmonella*. Retrieved from Mayo Clinic Web site <http://www.mayoclinic.com/health/salmonella/DS00926> on May 15, 2011
- McCullough, B. and Eisele, W. (1951): Experimental human salmonellosis. I. Pathogenicity of strains of *Salmonella meleagridis* and *Salmonella anatum* obtained from spray-dried whole egg. *J. Infect. Dis.*, **88**: 278-289.
- Mead, P., Slutsker, L., Dietz, V., McCaig, L., Bresee, J., Shapiro, C., Griffin, P. and Tauxe, R. (1999): Food-related illness and death in the United States. *Emer. Infect. Dis.*, **5**: 607-625.
- Mi-Kyung, P. (2009): Development of microscopic imaging system for rapid detection of *Salmonella* in raw chicken. A Dissertation paper for Degree of Doctor of Philosophy <http://etd.auburn.edu/etd/bitstream/handle/10415/1856/Dissertation%20whole%20part-final%20%28PDF%29.pdf?sequence=1> Accessed on May 15, 2011.
- MMWR Recomm Rep (2001): Diagnosis and management of food borne illnesses: a primer for physicians. January 26, 2001 / 50 (RR-2):1-69. Atlanta, GA: Centers for Disease Control and Prevention.

- Mohammed, A., Becker, H. and Terplan, G. (1996): Comparative studies on the detection of *Salmonellae* in Ethiopian cottage cheese (ayib) using different culture methods. *Arch. Lebensmittelhyg.*, **47**: 83-90.
- Molla, B., Kleer, J. and Sinell, J. (1999): Antibiotic resistance pattern of food borne *Salmonella* isolates in Addis Ababa (Ethiopia). *Berl. Munch. Tierarztl. Wochenschr.*, **112**: 41-43.
- Molla, B., Mohammed, A. and Salah, W. (2004): *Salmonella* prevalence and distribution of serotypes in apparently healthy slaughtered camels (*Camelus dromedarius*) in Eastern Ethiopia. *Trop. Anim. Hlth. Prod.*, **36**: 451-458.
- Molla, B., Salah, W., Alemayehu, D. and Mohamed, A. (2006): Antimicrobial resistance pattern of *Salmonella* serovars isolated from apparently health slaughtered Camels (*Camelus dromedarius*) in Eastern Ethiopia. *Berliner und Munchener Tierarztliche Wochenschrift*, **11**: 39-45.
- Murray, P., Baron, E. and Jorgensen, J. (2003): Manual of clinical microbiology, 8th Edition American society of Microbiology, Washington DC.
- O'Connor, C. (1993): Traditional cheese making manual. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia. ISBN: 92-9053-273-4.
- O'Mahony, F. (1988): Rural Dairy Technology - Experience in Ethiopia. ILCA Manual No. 4. International Livestock Center for Africa, Addis Ababa, Ethiopia.
- O'Mahony, M., Cowden, J. and Smyth, B. (1990): An outbreak of *Salmonella* saintpaul infection associated with bean sprouts. *Epid. Infect.*, **104**: 229-235.
- Old, C. and Threlfall, J. (1998): *Salmonella*. In: Topley and Wilson's Microbiology and Microbial Infections, 9th Edition. Systematic bacteriology, **2**, London: Pp. 969-991 Developing World Atlanta, Georgia, USA. Pp. 151-152.
- Pal, M. (2007): Zoonoses, 2nd Edition, Satyam Publishers, Jaipur, India. Pp.133-134.

- Palmera-Suárez, R., García, P., García, A., Barrasa, A. and Herrera, D. (2007): *Salmonella* Kottbus outbreak in infants in Gran Canaria (Spain) caused by bottled water, August-November 2006. *Euro Surveill*; **12**: E070712.2.
- Popoff, Y., Bockemühl, J. and Gheesling, L. (2004): Supplement 2002 (no. 46) to the Kauffmann-White scheme. *Res. Microbiol.*, **155**: 568-570.
- Schneitz, C. and Hakkinen, M. (1998): Comparison of two different types of competitive exclusion products. *J. Appl. Microbiol.*, **26**: 338-341.
- Scott, F., Threlfall, J. and Arnold, C. (2002): Genetic structure of *Salmonella* revealed by fragment analysis. *Internat. J. Syst. Evolution, Microbiol.*, **52**: 1701-1713.
- Shelobolina, S., Sullivan, A., O'Neill, R., Nevin, P. and Lovley, R. (2004): Isolation, characterization, and U (VI)- reducing potential of a facultatively anaerobic, acid-resistant bacterium from low-pH, nitrate- and U(VI)- contaminated subsurface sediment and description of *Salmonella subterranea* sp. nov. *Appl. Environ. Microbiol.*, **70**: 2959-2965.
- Smith, T. (1894): The hog-cholera group of bacteria. *U.S. Bur. Anim. Ind. Bull.*, **6**: 6-40.
- Su, H. and Chiu, H. (2007): *Salmonella*: clinical importance and evolution of nomenclature. *Chang. Gung. Med. J.*, **30**: 210-219.
- Sumner, J., Raven, G. and Givney, R. (2004): Have changes to meat and poultry food safety regulation in Australia affected the prevalence of *Salmonella* or of Salmonellosis? *Internat. J. Food Microbiol.*, **92**: 199-205.
- Ten years of experience of *Salmonella* infections in Cambridge, UK. *J. Infect.* **60**: 21-25.
- Tindall, J., Grimont, D., Garrity, M. and Euzéby, P. (2005): Nomenclature and taxonomy of *Salmonella*. *Internat. J. Syst. Evolution. Microbiol.*, **55**: 521-524.
- Todd, D. (1997): Epidemiology of food borne diseases: a worldwide review. *World Health Stat Q.*; 50 Pp 30-50.

- Tolga, K., Gursel, O., Basak, O. and Ergun, G. (2010): Prevalence of *Salmonella* spp. and *Listeria monocytogenes* in different cheese types produced in Turkey. *Bri. Food J.*, **112**: 1230-1236.
- Thrusfield, M. (2007): Veterinary epidemiology, 3rd Edition. Blackwell Science, UK. P. 332.
- UK Zoonoses Report (2000): Department for environment, food and rural affairs. http://www.defra.gov.uk/animalh/diseases/zoonoses/zoonoses_reports/zoonoses_2000.pdf (Accessed on 04/10/2006) 81
- Unnevehr, J. (2003): Food safety in food security and food trade. An overview: 2020 vision for food, agriculture and the environment. Focus 10, brief 1 of 17.
- USDA (1998): Progress Report: Pathogen Reduction Performance Standards for *Salmonella*, September 1998.
- Vaillant, V., de Valk, H., Baron, E., Ancelle, T., Colin, P. and Delmas, M. (2005): Food borne infections in France. *Food borne Pathog. Dis.*, **2**: 221-232.
- Van Cauteren, D., Jourdan-da, Silva, N., Weill, F., King, L., Brisabois, A., Delmas, G., Vaillant, V. and de Valk, H. (2009): Outbreak of *Salmonella enterica* serotype Muenster infections associated with goat's cheese, France, March 2008. *Euro Surveill.* **14** (31): pii =19290. Available online <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19290>
- Van Kessel, J., Karns, J. and Gorski, L. (2004): Prevalence of *Salmonellae*, *Listeria monocytogenes* and fecal coliforms in bulk tank milk on U.S. dairies. *J. Dairy Sci.*, **87**: 2822-2830.
- Veen, V. (2005): International trade and food safety in developing countries. *Food Cont.*, **16**: 491-496.
- Velge, P., Cloeckart, A. and Barrow, P. (2005): Emergence of *Salmonella* epidemics: The problem related to *Salmonella enterica* serotype Enteritidis and multiple antibiotic resistance in other major serotypes. *Vet. Res.*, **36**: 267-288.

- Victoria, G., O'Reilly, M., Carsten, M., Ian, W. and David, L. (2006): Low tyrosine content of growth media yields aflagellate *Salmonella enterica* serovar Typhimurium. *Microbiology*, **152**: 23-28.
- Voetsch, A., Van Gilder, T., Angulo, F., Farley, M., Shallow, S. and Marcus, R. (2004): Foodnet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clin. Infect. Dis.*, **38**: 127-134.
- Warnick, L., Kaneene, J. and Ruegg, P. (2003): Evaluation of herd sampling for *Salmonella* isolation on Midwest and northeast U.S. dairy farms. *Prev. Vet. Med.*, **60**: 195-206.
- Weir, E., Dore, K. and Currie, A. (2004): Enhanced surveillance for *Salmonella* Newport. *Can. Med. Assoc. J.*, **171**: 127-128.
- WHO and CDC, World Health Organization and centre for disease control and prevention (2003): Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance.
- WHO, World Health Organization (1999): Food Safety Programme “ Food Safety – An Essential Public Health Issue for the New Millenium”, (WHO/SDE/PHE/FOS/99.4), hereafter “*Food Safety An Essential Public Health Issue for the New Millennium*. P. 7.
- WHO, world health organization (2009): Global tuberculosis Control WHO Report 2009 epidemiology, strategy, financing, WHO Press World Health Organization Geneva, Switzerland.
- Wolday, D., Erge, W. (1998): Antimicrobial sensitivity pattern of *Salmonella*: comparison of isolates from HIV-infected and HIV uninfected patients. *Trop. Doct.*, **28**:139-141.
- Wood, S., Collins-Thompson, L., Irvine, M. and Myhr, N. (1984): Source and persistence of *Salmonella* Muenster in naturally contaminated Cheddar cheese. *J. Food Protect.*, **47**: 20-22.

Xanthopoulos, V., Polychroniadou, A., Litopouloutzanetaki, E. and Tzanetakis, N. (2000): Characteristics of Anevato cheese made from raw or heat-treated goat milk inoculated with a lactic starter. *Lebensm.-Wiss. Technol.*, **33**: 483-488.

Yagoub, O., Oshi, M. and El-Zubeir, M. (2006): Isolation and susceptibility to antimicrobial agents of *Salmonella paratyphi* from cheese in Khartoum (Sudan). *Res. J. Microbiol.*, **1**: 110-114.

Zhao, S., Qaiyumi, S., Friedman, S., Singh, R., Foley, S., White, D., McDermott, P., Donkar, T., Bolin, C., Munro, S., Baron, E. and Walker, R. (2003): Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. *J. Clin. Microbiol.*, **41**: 5366-5371.

8. ANNEXES

Annex 1. Sample collection record sheet for bacteriological analysis

Serial No.	Source	Status of sample	Storage time	Remark
1				
2				
3				
5				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				

Annex 2. Record sheets for laboratory analysis of *Salmonella*

S. no.	Growth on		Typical <i>Salmonella</i> Colony	Biochemical test result									Growth on chromogenic media	<i>Salmonella</i> positive	Remark	
	XLD agar	SSA agar		TIS				LDC			VP test	Urea test				Indole test
				slant	butt	H ₂ s	Gas	Slant	butt	H ₂ s						

Annex 3. Composition and preparation of culture media and reagents used for the laboratory analysis of the study sample

A. non selective pre enrichment Buffered peptone water (BPW) (AES laboratoire, Codex, France)

Compositions	g/l
Peptone	10
Sodium Chloride	5
Disodium Phosphate	3.5
Monopotassium Phosphate	1.5
Final pH: 7.2 ± 0.2 at 25°C	

Appearance: Pale straw, clear liquid.

Directionss

- Dissolve 20 g of the medium in one liter of purified water.
- Heat with frequent agitation to completely dissolve the medium, if necessary
- Autoclave at 121°C for 15 minutes

B. Selective enrichment rappaport-vassiliadis (RV) broth (Oxoid Ltd., Basingstoke Hampshire, England)

Compositions	g/l
Soya peptone	5.0
Sodium chloride	8.0
Potassium dihydrogen phosphate	1.6
Magnesium chloride $6\text{H}_2\text{O}$	40.0
Malachite green	0.04
pH 5.2 ± 0.2 @ 25°C	

Directions

Add 30 g (the equivalent weight of dehydrated medium per liter) to 1 liter of distilled water. Heat gently until dissolved completely. Dispense 10 ml volumes into screw-capped bottles or tubes and sterilize by autoclaving at 115 °C for 15 minutes.

C. Selective and non selective media used for plating of the target pathogen

- i. Nutrient agar used for non selective plating (Oxoid Ltd., Basingstoke Hampshire, England)

Compositions	g/l
Yeast extract	4.0
Tryptone	5.0
Glucose	50.0
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol green	0.022
Agar	15.0
pH 5.5 ± 0.2	

Directions

- Suspend 75 g in 1 liter of distilled water. Bring to the boil to dissolve completely.
- Sterilize by autoclaving at 121 °C for 15 minutes.
- If required the pH may be adjusted to 6.5 by the addition of 1% sodium bicarbonate solution.

ii. *Salmonella Shigela* (SS) agar (Difco™, Detroit, USA)

Compositions	g/l
Beef Extract	5.0
Proteose Peptone	5.0
Lactose	10.0
Bile Salts No. 3	8.5
Sodium Citrate	8.5
Sodium Thiosulfate	8.5
Ferric Citrate	1.0
Agar.	13.5
Brilliant Green	0.33 mg
Neutral Red	0.025

Directions

- Suspend 60 g of the powder in 1 liter of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Do not autoclave.
- Cool the medium to approximately 45-50 °C and pour into Petri dishes.
- Allow the plates to dry for approximately 2 hours with the covers partially removed.
- Test samples of the finished product for performance using stable, typical control cultures.

iii. Xylose Lysine Desoxycholate (XLD) agar (AES laboratoire, Cedex, France)

Compositions	g/l
Yeast extract	3.0
L-Lysine HCl	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5
pH 7.4 ± 0.2	

Directions

- Suspend 57 g of dehydrated medium in 1 liter of distilled or deionized water.
- Heat to about 90 °C with frequent agitation
- Stop heating when the medium is completely dissolved.
- Do not autoclave.
- Cool in a water bath to 45/50 °C.
- Swirl or stir gently to homogenize. Pour into sterile Petri dishes and allow to gel and dry.
- If not used soon, store in the dark. Prepared media plates can be kept for one day at ambient temperature. Plates can be stored for up to two weeks under refrigeration (2-8 °C) if properly prepared and protected from light and dehydration.

iv. CHROMagar™ *Salmonella* Plus (DRG New Jersey, USA)

Compositions	g/l
Agar	15.0
Peptone & Yeast extract	8.0
Salts	8.5
Chromogenic mix	1.3
Supplement	6.0
pH : 7.6 +/- 0.2	

Directions

- For pre-weighed dose of medium, add dry powder to the corresponding volume of purified water. Alternatively, suspend the medium in the proportion of 32.8 g per liter of purified water.
- Disperse the powder slowly in water by rotating for swelling of the agar.
- Add the liquid content of the supplement vial in the proportion of 6 g/L.
- Swirl for mixing, Heat and bring to boiling (100 °C) while swirling or stirring regularly.
- If using an autoclave, do so without pressure. Do not heat to more than 100 °C.
- The mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently.
- Then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).
- Cool in a water bath to 45/50 °C.
- Swirl or stir gently to homogenize. Pour into sterile Petri dishes and allow to gel and dry.
- If not used soon, store in the dark. Prepared media plates can be kept for one day at ambient temperature. Plates can be stored for up to two weeks under refrigeration (2-8°C) if properly prepared and protected from light and dehydration.

D. Media used for biochemical analysis of suspected colonies after plating on nutrient agar

i. Triple sugar iron agar (TSI) (Oxoid, Oxoid Ltd., Basingstoke Hampshire, England)

Compositions	g/l
Lab-Lemco powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0

PH 5.2 ± 0.2 at 25 °C

Directions

Suspend 65 gm in 1 liter of distilled water. Bring to the boil to dissolve completely. Mix well and distribute. Sterilize by autoclaving at 121 °C for 15 minutes. Allow the medium to set in sloped form with a butt about 1 inch deep.

ii. SIM Medium (BBL™) for indole test (Becton Dickinson and company Cockeysville, USA)

Compositions	g/l
Pancreatic Digest of Casein	20.0
Peptic Digest of Animal Tissue	6.1
Ferrous Ammonium Sulfate	0.2
Sodium Thiosulfate	0.2
Agar	3.5

Directions

- Suspend 30 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Dispense and autoclave at 121 °C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

iii. Kovacs Reagent (Oxoid Ltd., Basingstoke Hampshire, England)

Composition	g /l
p-Dimethylaminobenzaldehyde	50.0 g
Amyl Alcohol	750.0 ml
Hydrochloric Acid	250.0 ml

- iv. Lysine iron agar (BBL™) for Lysine decarboxylase (LDC) test (Becton Dickinson and company Cockeysville, USA)

Compositions	g/l
L-Lysine	10.00
Yeast Extract	3.00
Ferric Ammonium Citrate	0.50
Bromocresol Purple	0.02
Gelatin Peptone	5.00
Dextrose	1.00
Sodium Thiosulfate	0.04
Bacteriological Agar	13.50

Directions

Suspend 33 grams of the medium in one liter of distilled water. Mix well and dissolve while heating and boil for one minute. Dispense in tubes and sterilize at 121 °C for 12 minutes. Cool in a slanted position.

- v. Methyl red- Voges–Proskauer (MR-VP) medium for Voges–Proskauer VP test (Titan Biotech Ltd. Bhiwadi, India)

Compositions	g/l
Enzymatic Digest of Casein	3.5
Enzymatic Digest of Animal Tissue	3.5
Dextrose	5
Potassium Phosphate	5
Final pH: 6.9 ± 0.2 at 25°C	

Directions

- Dissolve 17 g of the medium in one liter of purified water.
- Heat with frequent agitation and boil for one minute to completely dissolve the medium.
- Autoclave at 121 °C for 15 minutes.

vi. Reagents used for VP test (40% potassium hydroxide and 1- alpha-naphthol)

Compositions

Potassium Hydroxide (40%)

Potassium Hydroxide	400.0 g
Sterile De-ionized Water	1000.0 ml

1- alpha- naphthol

1-naphthol	60.0 g
96% (volume fraction) of ethanol	1000.0 ml

Directions

Dissolve the 1-naphthol in the ethanol alcohol and Potassium hydroxide in distilled water.

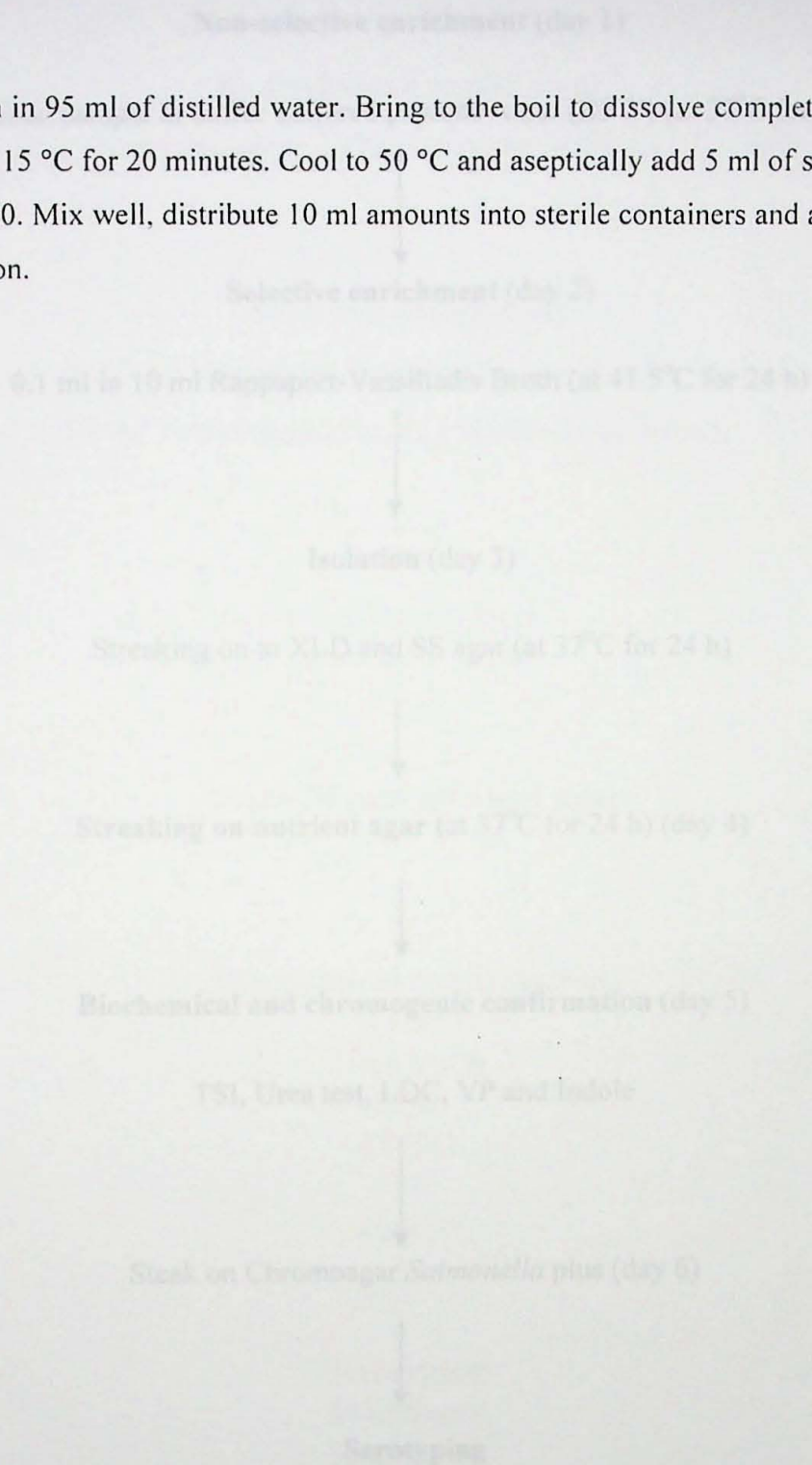
vii. Urea agar base (Code: CM0053) for urea test (oxoid Ltd., Basingstoke Hampshire, England)

Composition	g/l
Peptone	1.0
Glucose	1.0
Potassium dihydrogen phosphate	0.8
Disodium phosphate	1.2
Sodium chloride	5.0
Phenol red	0.012
Agar	15.0
PH 6.8 ± 0.2	

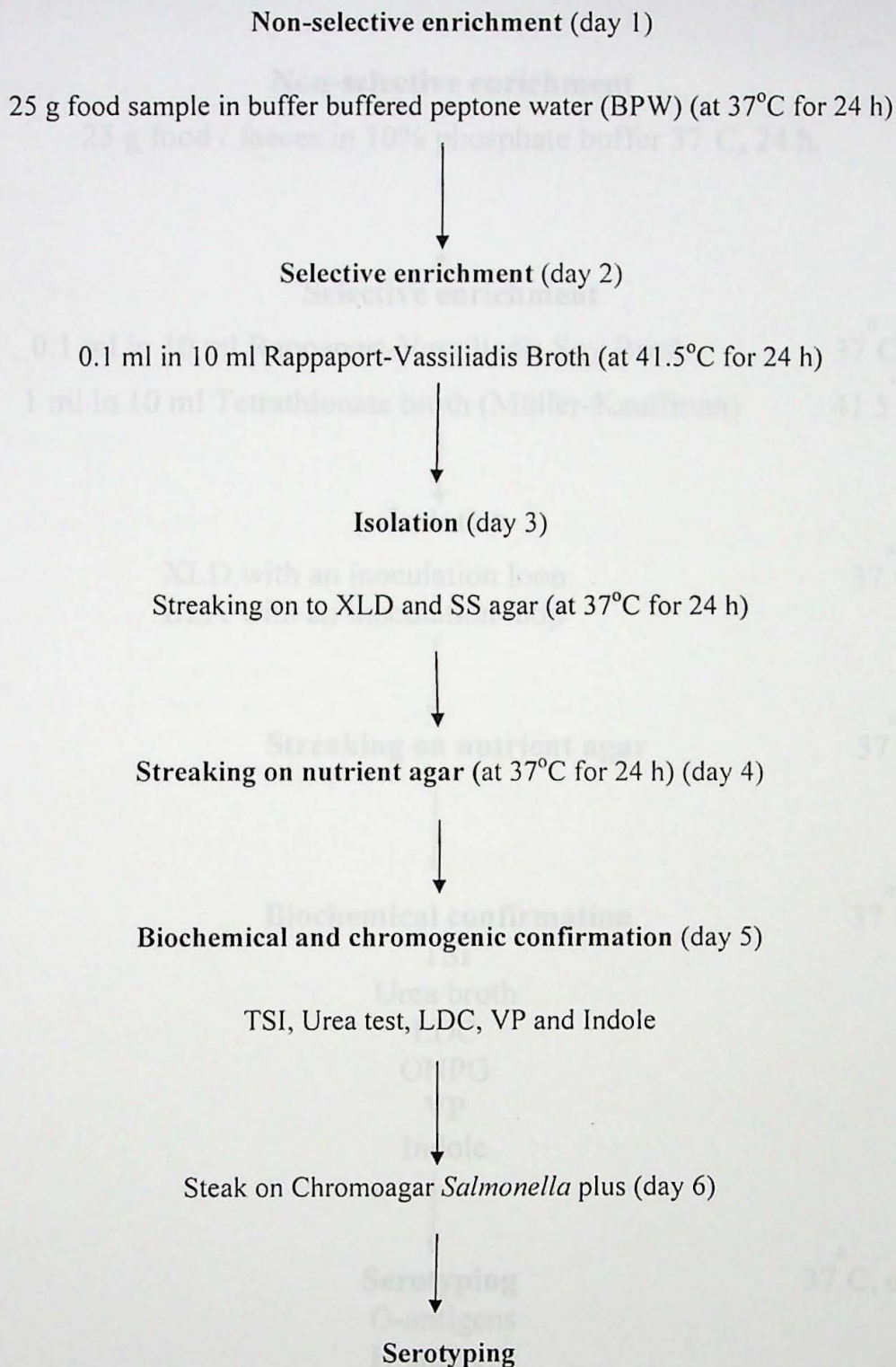
Supplement 40% Urea Solution (SR0020) oxid have been used 5 ml for 95 ml of urea agar base

Directions

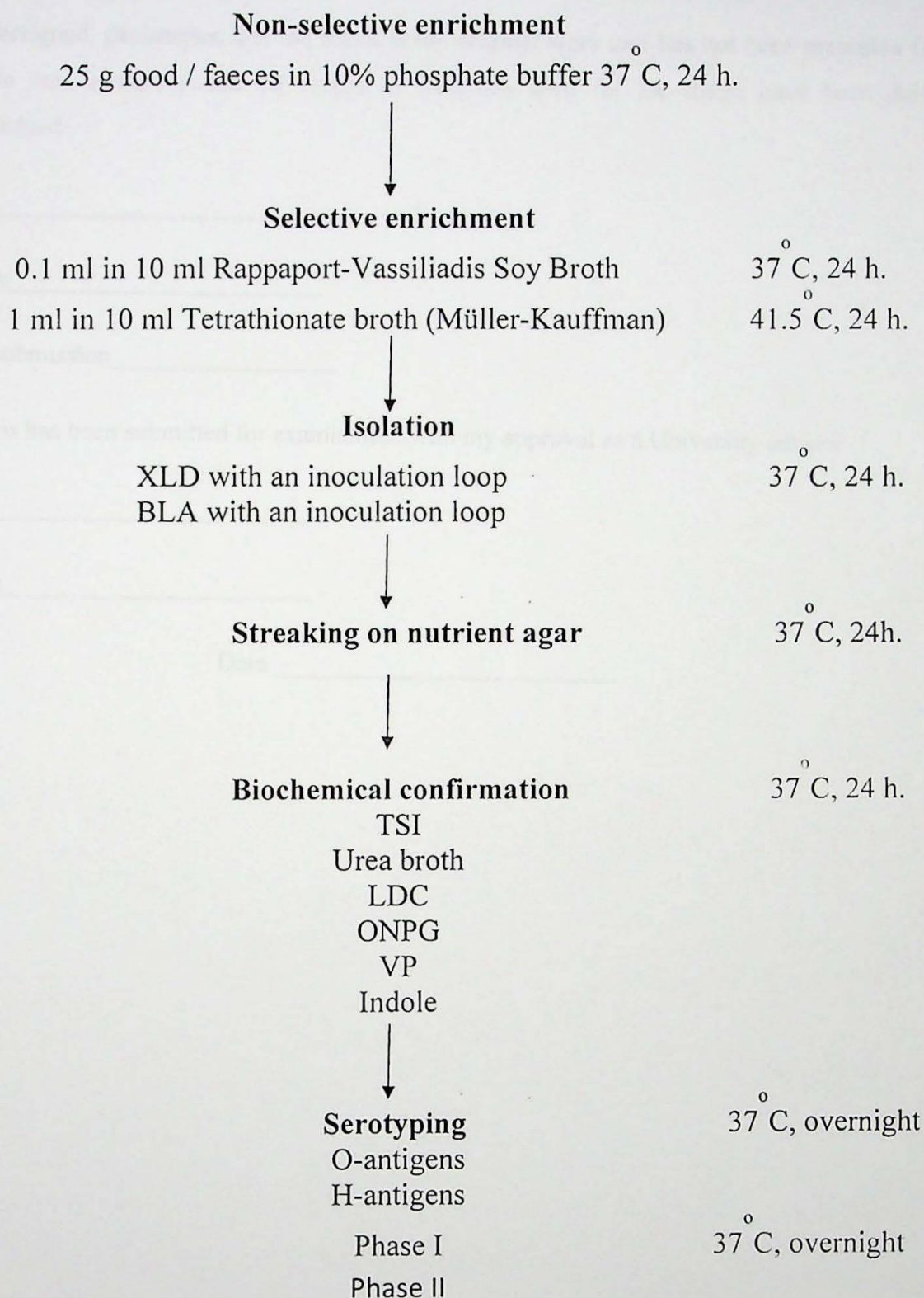
Suspend 2.4 gm in 95 ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115 °C for 20 minutes. Cool to 50 °C and aseptically add 5 ml of sterile 40% Urea Solution SR0020. Mix well, distribute 10 ml amounts into sterile containers and allow cooling in the slope position.



Annex 4. Flow diagram for isolation and identification of *Salmonella* used in this study



Annex 5. Flow diagram for detection of *Salmonella* (Global Salm-Surv, 2003)



9. SIGNED DECLARATION SHEET

The undersigned, declaration that the thesis is my original work and has not been presented for degree in any university and all source of materials used for the thesis have been dully acknowledged.

Name _____

Signature _____

Date of submission _____

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

Name _____

Signature _____

Date _____