Thesis Ref. No. _____

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBLITY PROFILE OF *SALMONELLA* IN SLAUGHTERED CATTLE AND LACTATING COWS IN CENTRAL ETHIOPIA

MSc THESIS

BY

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DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND VETERINARY PUBLIC HEALTH

MSc PROGRAM IN VETERINARY MICROBIOLOGY

October, 2014

College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBLITY PROFILE OF *SALMONELLA* IN SLAUGHTERED CATTLE AND LACTATING COWS IN CENTRAL ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Microbiology

By

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October, 2014

College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by Bulako Chebo entitled Prevalence and Antimicrobial Susceptibility Profile of *Salmonella* in Slaughtered Cows and Lactating Cows in Central Ethiopia that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Veterinary Microbiology.

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TABLE OF CONTENTS **PAGES**

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my academic advisors Dr. Bedaso Mamo and Dr. Ashenafi Feyissa for over all guidance and encouragement during the research work.

I am deeply grateful to Dr. Fufa Abuna and Dr. Reta Duguma, the leaders of thematic research on *Salmonella* and *Staphylococcus* for letting me work in their project, material and financial support.

I wish to express my sincere thanks to members of Addis Ababa, Bishoftu, Adama, Holeta and Asella municipal abattoir workers for their help and cooperation during sample collection. I also would like thank the diary farm owners who allowed sampling in their farms and attendants for cooperation in sample collection.

Staffs of the microbiology laboratory at CVMA and Asella regional veterinary diagnostic laboratory deserve special appreciation for their support, understanding and tolerance during the height of our work in the laboratory.

LIST OF ABBREVATIONS

AML	Ampicilline
AMP	Amoxicillin
A_W	Water activity
BGA	Brilliant green agar
BPW	Buffered peptone water
BSA	Bismuth sulfite agar
C	Chloramphenicol
CDC	Center for Disease Control
CI	Confidence interval
CIP	Ciprofloxacin
CN	Gentamycin
CVMA	College of Veterinary Medicine and Agriculture
DC	Dendritic cells
FAO	Food and Agricultural Organization
FOX	Cefoxitine
gms	Grams
H	Flagellar antigen
HACCP	Hazard analysis critical control point
HeK	Hektoen enteric
hrs	Hours
H_2S	Hydrogen sulfide
ISCOM	Immunostimulating complex
ISO	International Organization for Standardization
K	Kanamycin

Km	Kilometers
LIA	Lysine iron agar
LPS	Lipopolysaccharide
ml	Milliliters
O	Somatic antigen
OIE	Office International des Epizooties
MR	Methyl red
OMP	Outer membrane proteins
OR	Odds ratio
S	Streptomycin
SCV	*Salmonella* containing vesicles
SIF	*Salmonella* induced filaments
SPIs	*Salmonella* Pathogenecity Islands
Spp	Species
SS	*Salmonella- Shigella*
SXT	Trimethoprim-Sulfamethoxazole
TSI	Triple sugar iron
T3SS	TypeIII secretion System
USDA	United States Department of Agriculture
VP	Vogus-Proskauer
XLD	Xylose-lysine-deoxycholate
WHO	World Health Organization

LIST OF TABLES **PAGES**

LIST OF FIGURE **PAGE**

LIST OF APPENDICES **PAGES**

ABSTRACT

The survey was conducted on 133 apparently healthy slaughtered cattle and 202 lactating dairy cattle at selected towns of central Ethiopia (Addis Ababa, Bishoftu, Adama, Holeta and Asella) from December 2013 to June 2014. The objectives were to estimate the prevalence and distribution of *Salmonella* in lactating cows and slaughtered cattle, Assess the antimicrobial resistance pattern of *Salmonella* isolates and find out some of the risk factors of milk and carcass contamination. A total of 984 samples from farms and abattoirs consisting of udder milk, tank milk, pooled buckets swab, pooled tank swab, feces from lactating animals, milkers' hand swab, carcass swab, cecal content from slaughtered animals, mesenteric lymph node, pooled butchers' hand swab, pooled carcass hanging materials swab and pooled knives swab were collected. The samples were examined for the presence of *Salmonella* following standard techniques and procedures outlined by the international organization for standardization. In a total of 335 animals examined, 29 (8.7%) were positive of these, 13(9.8%) were slaughtered cattle and 16 (7.92%) were lactating cows. In a total of 984 different samples *Salmonella* was isolated 66(6.71%). In 545 samples originated from farms *Salmonella* was isolated in 26(4.77%) of which 4(1.91%) was udder milk, 1(2.94%) tank milk, 1(2.94%) pooled buckets swab, 3(8.82%) pooled tank swab, 16(7.92%) feces from lactating animals and 1(2.94%) milkers' hand swab. From 439 samples collected from abattoirs *Salmonella* was isolated in 40(9.11%) of which 13(7.10%) carcass swab, 7(5.34) cecal content from slaughtered animals, 6(12.72%) mesenteric lymph node, 4(15.38%) pooled butchers' hand swab, 5(23.80%) pooled carcass hanging materials swab and 5(17.85%) pooled buchers' knives swab. *Salmonellae* were detected from all test samples obtained from farms and abattoirs. Up on assessment of association of milk contamination with risk factors, carrier animals and *Salmonella* contaminated tanks were found to be potential risk factors. Up on assessment of carcass contamination with potential risk factors, carcass hoisted on *Salmonella* positive hanging materials was found to be four times more likely to be contaminated with *Salmonella* compared to carcass hoisted on *Salmonella* negative hanging materials. Total 66 isolates obtained from the study were tested for ten different antimicrobials that were commonly used in human as well as

animal treatment, and available in the market. 56 (84.8%) were resistant to one or more of the tested antimicrobials. Of the resistant isolates, 87.5% (49 of 56) of them developed resistant to more than one drug.

Key words: *Abattoir, Antimicrobial resistance, Central Ethiopia, Farm, Lactating cow, Prevalence, Salmonella, Slaughtered cattle*

1. INTRODUCTION

Food safety has been a concern of mankind since the dawn of history. Despite advance in food science and technology, food borne diseases are among the most widely spread global public health problems of recent times and their implication for health and economy is increasingly recognized. The world Declaration on Nutrition adopted by FAO/WHO International Conference on Nutrition emphasizes that hundreds of millions of people suffer from communicable and non-communicable diseases caused by contaminated food and water (Zewdu, 2004; Busani *et al.*, 2005; Teklu, 2008).

Wide spectrum of pathogens play major role in causing food borne diseases. Most of them are zoonotic and have reservoirs in healthy food animals from which they spread to variety of foods. Therefore food of animal origin is considered as major vehicles of food borne infections (Buzby and Roberts, 2009). Salmonellosis is one of the most common and widely distributed food borne diseases associated with food of animal origin and is caused by the bacteria *Salmonella*. WHO (2007) reports that, *Salmonella* covers 88% of the food borne infections. In many registers non-typhoidal *Salmonella* species are documented as one of the leading causes of bacterial diseases. Food borne *Salmonella* typically causes acute gastroenteritis and may cause a more septicemic disease usually in very young, the elderly and immunocopromised subjects (Teklu, 2008).

Salmonella species occur widely in natural environment and in different sectors of the global food chain. The ability of these microorganisms to survive under adverse conditions and to grow in the presence of low level of nutrients and at sub optimal temperatures and PH values presents a formidable challenge to the agriculture and food processing industries in marketing safe products. The continued prominence of raw meats, eggs, dairy products, vegetable sprouts, fresh fruits, and fruit juices as the principal vehicle of human food borne salmonellosis arises from major difficulties to coordinate sectorial control efforts within each industry. The problem of salmonellosis is further compounded by the massive and unrestricted movement of food in international

trade, the national disparities in the hygienic agricultural and aqua cultural production of foods and the non-uniform government and industry food safety controls during production of foods and the non-uniform government and industry food safety controls during the processing, distribution and marketing of fresh and processed food products (Molla *et al*., 2003a; Teklu, 2008).

Moreover, antimicrobial resistance is currently the greatest challenge to the effective treatment of infections globally and more than 80% of food poisoning bacteria such as *Salmonella* are reported as antibiotic resistant to at least one type of antimicrobial and more than 50% are resistant to two or more and are major public health concerns (Teshome and Anbessa, 2012). The use of antibiotics during animal production was the main reason for the development of antimicrobial resistant *Salmonella* species. Globally, the three main causes of antimicrobial resistance have been identified as use of antimicrobial agents in agriculture, over-prescribing by physicians and misuse by patients (Dabassa and Bacha, 2012). Routine assessment of patterns of emerging antibiotic resistant *Salmonella* strains is of principal importance because such information channeled to physicians and veterinarians help to timely redirect drug use so as to diminish the development and spread of resistance.

Food animals harbor a wide range of *Salmonella* serotypes and so act as a source of contamination, which is of paramount epidemiological importance in non-typhoidal human salmonellosis (Teklu, 2008). Cattle can be chronically infected and serve as carriers within the herd without exhibiting clinical signs. It has been reported that one carrier cow can shed one billion *Salmonellae* a day in the feces. *Salmonella* shed in the feces of livestock such as cows and goats can contaminate milk during the milking process (Randall, 2001). The Garment, fecal wastes from infected animals, storage material and ways of handlings are important sources of *Salmonella* contamination of the raw milk. Humans and other animals can become infected from consumption of contaminated drinking water, raw dairy and milk products, and undercooked meat products (Teshome and Anbessa, 2012). Despite the presence of many studies on the prevalence and antimicrobial susceptibility pattern of *Salmonella* in Ethiopia both in

veterinary and public health setups, reports on prevalence and antimicrobial resistance profile of *Salmonella* in apparently healthy lactating cows and cows' raw milk is very limited.

Cross-contamination of carcasses with *Salmonella* can also occur during slaughtering operations. Stress associated with transport of animals to abattoir augments shedding of *Salmonella* by carrier animals and this may contribute to the spread of the organism to other animals in the slaughter plant (Isaacson *et al*., 1999). Slaughtering procedures potentially involve many risks of both direct and cross-contamination of carcasses and meat surfaces. During slaughter, faecal contamination of edible organs with subsequent contamination of the carcass may occur. This can be carried through all slaughter procedures up to the processing of the raw products, which are important sources of *Salmonella* in the human food chain. Contamination of equipment, utensils and hands of workers can spread *Salmonella* to uncontaminated carcasses and parts, which can occur in subsequent handling, processing, transport, storage, distribution and preparation for consumption (Ejeta *et al*., 2004).

There are a number of published information in Ethiopia on salmonellosis indicated the existence of infection in various animals species (poultry, camel, cattle, swine, sheep and goats), retailed food items (minced beef, chicken meat and offals) as well as humans (Nyeleti *et al*., 2000; Woldemariam *et al*., 2005; Molla *et al*, 2006; Teklu, 2008; Legesse *et al*., 2014). However, integrated information on cattle salmonellosis covering wider area is not available. In addition, a periodic surveillance of the level of *Salmonella* contamination in the different food animals, food products and environment is necessary to control the spread of the pathogen and infection of man.

Hence this cross sectional study was designed to study salmonellosis in apparently healthy slaughtered cattle and lactating cows at central Ethiopia as part of generating base line information covering wider areas with the following specific objectives;

- To estimate the prevalence and distribution of *Salmonella* in lactating cows and slaughtered cattle;

- To assess the antimicrobial resistance pattern of *Salmonella* isolates; and
- To find out some of the risk factors of milk and carcass contamination

2. LITRATURE REVIEW

2.1. Historical prospective

The genus *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist. While Theobald Smith was the actual discoverer of the type bacterium ("*Salmonella enterica*" var. Choleraesuis) in 1885 from porcine intestine. Dr. Salmon was the administrator of the United States Department of Agriculture (USDA) research program, and thus the organism was named after him. Smith and Salmon had been searching for the cause of common hog cholera and proposed this organism as the causal agent. Later research, however, would show that this organism (now known as *Salmonella enterica*) rarely causes enteric symptoms in pigs, and was thus not the agent they were seeking (which was eventually shown to be a virus). However, related bacteria in the genus *Salmonella* were eventually shown to cause other important infectious diseases (http://www.news-medical.net/health/Salmonella-History.aspx). The genus *Salmonella* was finally formally adopted in 1900 by J. Lignieres for many species of *Salmonella*, after Smith's first type species *Salmonella* Choleraesuis (Girmay, 2013).

The first report of laboratory-confirmed outbreak of food borne salmonellosis described an episode in which 58 persons in 25 different families who had eaten beef developed acute gastroenteritis; one died. Gartner isolated the 'Gartner-bacillus' from infected cow from which the meat came, and from organ of fatal case. Kauffman determined that the 'Gartner-bacillus' from this outbreak was serotype Enteritidis, but outbreak of 'Gartner-bacillus' was of serotype Dublin and possibly other serotypes. Mice, rabbit, goat guinea pig were affected when inoculated with the bacillus. In the following years, several out breaks of salmonellosis affecting man or animals were reported and old concept of 'meat poisoning' was linked with the etiologic agent *Salmonella*. Subsequently, human salmonellosis occurred primarily among individuals who ate meat from ill animals, mainly cattle, but also pigs or goats (MØlbak *et al*., 2006; Teklu, 2008).

The development of serotyping was fundamental for the understanding of the epidemiology of *Salmonella* infections. While *S.* Typhi was easy to recognize by biochemical tests, it was for example, impossible to distinguish between *S.* Typhimurium and *S.* Paratyphi B on the basis of fermentation of sugars or other biochemical properties. Many bacteriologists considered *S.* Typhimurium and *S.* Paratyphi B to be identical. However, it was an enigma why infection seemingly identical bacteria often did not share pathological, clinical and epidemiological features. This and several other questions were resolved at the end of the 1920s when White and Kauffman, through the use of improved serological methods, succeeded in designing a classification system for *Salmonella*. The foundation for this serotyping scheme was the discovery of the flagellar (H) antigen and the thermostable somatic O antigen by Weil and Felix and the phase shift in the H antigen (D'Aoust, 1997; MØlbak *et al.*, 2006).

2.2. Taxonomy and nomenclature

Salmonella nomenclature is complex, and scientists use different systems to refer to and communicate about this genus. However, uniformity in *Salmonella* nomenclature is necessary for communication between scientists, health officials, and the public. Unfortunately, there was usage often combines several nomenclatural systems that inconsistently divide the genus into species, subspecies, subgenera, groups, subgroups, and serotypes (serovars), and this causes confusion. Centre for Disease Control (CDC) receives many inquiries concerning the appropriate *Salmonella* nomenclature for the reporting of results and for use in scientific publications (Brenner *et al*., 2000).

According to the latest nomenclature, which reflects recent advancement in taxonomy, the genus *Salmonella* consists of three species (spp): *S. enterica*, the type species, *S. bongori*, former subspecies V and *S. subterranean* (Teklu, 2008). *S. enterica* consists of six subspecies which are referred by a roman numeral and a name: I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enteric* subsp. *houtenae*; and VI, *S. enterica* subsp. *indica*. These subspecies are further classified into more than 50 serogroups based on the O

antigen structure, which reflects variation in the exposed part of the lipopolysaccharide (LPS), and then further divided into > 2500 serovars based on the variation in flagellin (H antigen). Further classification in to serotypes (serovars) is done using the Kauffman-White scheme, which is defined and maintained by the WHO Collaborating Center for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France (Brandt, 2012). Subspecies I has far more serovars than the others (Brandt, 2012) and contains around 60% of known *Salmonella* serovars that inhabit the intestinal tract of humans and warm-blooded animals (Broz *et al*., 2010). Notwithstanding this, only a small fraction within subspecies I serovars are enteric pathogens; in fact according to CDC, United States of America, the 12 most prevalent *Salmonella* serovars have been shown to be responsible for more than 60% of all human *Salmonella* infections (Guma *et al*., 2009).

Serotypes of the other species are common in poikilothermic animals and in the environment, although some serotypes of *S. arizonae* and *S. diarzonae* have been associated with diseases in Turkey and sheep (Teklu, 2008).

CDC of the United States uses names for serotypes in sub species I (for example, serotypes Enteridis, Typhimurium, and Choleraesuis) whereas antigenic formula for unnamed serotypes described after 1996 in subspecies II, IV, and VI and in *S. bongori*. For named serotypes, to avoid confusion between serovars and species, the serovar name is not italicized and starts with a capital letter. When cited 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. Afterward the name may be shortened with the genus name followed directly by the serotype name, e.g., *Salmonella* Choleraesuis or *S*. Choleraesuis (CDC, 2011).

Abbreviating genus name with as ''*S*'' and omitting the species name followed by the serotype name (e.g., *S.* Typhimurium) has disadvantages: It can be misinterpreted as giving species/taxonomic standing to serotypes; it is formatted as a taxonomic designation. However, serotypes have no taxonomic standing, and this abbreviation is not correct taxonomically. To be taxonomically correct, the name must include species (e.g.,

S. enterica ser. Typhimurium); "*S."* could mean *Shigella*, *Serratia*, *Sutterella*, or any of a number of genera. This is most likely to be a problem with less common serotypes or when a variety of genera are being considered; "*S.* Cholerasuis" and "*S.* Enteritidis" were historically used as taxonomic species names. The incorrect abbreviations "*S.* Cholerasuis" and "*S.* Enteritidis", which are intended to denote serotypes, are difficult to differentiate from the historical species names, which are sometimes still used and found in the literature (CDC, 2011).

Serotype names designation by antigenic formulae include: Subspecies [space] O antigens [colon] Phase 1 H antigen [colon] Phase 2 H antigen. For example: I 4,5,12:i:1,2 (*S. enterica* serotype Typhimurium); I 4,12:i:1,2 (*S. enterica* serotype Typhimurium var. O:5-); I 9,12:g,m:- (*S. enterica* serotype Enteritidis); II 47:b:1,5 (*S. enterica* serotype II 47:b:1,5); IV 48:g,z51:- (*S. enterica* serotype IV 48:g,z51:-*);* IIIb 65:(k):z *(S. enterica serotype* IIIb 65:(k):z) (Grimont and Weill, 2007).

For formulae of serotypes in *S. bongori*, V is still used for uniformity (for example, S. V_{61}: Z_{35} :). Before 1996 all serotypes in all sub species except subspecies IIIa and IIIb were given names. In 1996 the WHO collaborating center began naming serotypes only in subspecies I and dropped all existing serotype names in sub species II, IV and VI and *S. bongori* from Kauffman- White scheme. For surveillance purpose i.e., for compatibility with old data, CDC continue to use pre-1996 names for serotypes in subspecies II, IV and VI and *S. bongori* (Brenner, *et al.*, 2000).

Salmonella serotypes are named according to the disease they cause (S. Enteritidis, S. Typhi, S.Paratyphi, S. Abortus equi and S. Bovismorbificans) or the animal from which they were isolated. For example, S. Gallinurum and S. Pullorum were important pathogens in poultry, S. Choleraesuis an important swine pathogen, and S. Typhimurium got its name because it was originally isolated from ill laboratory mice. A limited number of serotypes named after the person who isolated (e.g. S. Virchow). Currently each new antigenically distinguishable is typically named after the geographical place at which it was first isolated (Teklu, 2008).

2.3. Characteristics of *Salmonella*

The family *Enterobacteriaceae* consists of gram negative, facultatively anaerobic, non spore forming rods. *Salmonella* conforms to the general definition of the family. Members of the genus are motile by peritrichous flagellation except *S. enterica* ser. Pollorum and *S. enterica* ser. Gallinarum, which lack flagella. Non motile variants can also arise as result of a faulty assembly of flagellar subunits or deficiencies in the motor functions in these appendages. *Salmonellae* are chemoorganptrophic, with an ability to metabolize nutrients by the respiratory and fermentative pathway. An important characteristic of the *Salmonella* bacteria is that they are able to grow and multiply outside living host organisms, thus having greater survival chances than otherwise (Gray and Fedorka-Cray, 2002; Teklu, 2008).

2.3.1. Biochemical characteristics

Salmonellae are generally unable to ferment lactose, sucrose or salicin, although glucose and certain other monosaccharides are fermented with production of gas. They are usually catalase positive, oxidase negative, and reduce nitrates to nitrites. The organisms use citrate as the sole carbon source, decarboxylate lysine, arginine and ornithine and produce hydrogen sulphide. The methle red reaction is positive, the Vogus–Proskauer test is negative and indole is negative. Phenylalanine is not delaminated, urea is not hydrolysed, gelatin is not liquefied rapidly in nutrient media and neither DNAase nor lipases are produced. *Salmonellae* may harbor temperatures phages or plasmids that code for metabolic characters used in identification (e.g. H_2S, lactose or sucrose fermentation) (Coetzer *et al.*, 1994; D'Aoust, 1997; 2000; Jay, 2000; MØlbak *et al.*, 2006).

2.3.2. Growth and physical characteristics

Salmonellae are able to grow on a large number of culture media and produce visible colonies well within 24 hours at about 37^{o}C. The parameters of pH, water activity (a_w), nutrient content and temperature are all interrelated for *Salmonellae,* as they are for most

other bacteria. The pH for optimum growth is around neutrality (between 6.6 and 8.2), with value above 9.0 and below 4.0 being bactericidal. A minimum growth pH of 4.05 has been recorded for some (with hydrochloric and citric acids), but depending on the acid used to lower the pH, the minimum may be as high as 5.5. Aeration was found to favor growth at lower pH values (Jay, 2000; D'Aoust, 2001).

Salmonellae grow in the temperature range of 2- 47 ^{o}C with rapid growth between 25 and 43^{o}C (D'Aoust, 2000; 2001). The lowest temperature at growth has been reported are 5.3^{o}C for S. Heidelberg and 6.2 ^{o}C for S. Typhimurium. Temperature around 45^{o}C has been reported by several investigators to be upper limit for growth. Regarding available moisture, the growth inhibition has been reported for a_w value below 0.94 in media with neutral pH, with a_w value being required as the pH is decreased toward growth minima (Coetzer *et al*., 1994; Jay, 2000; D'Aoust, 2001).

Compared to other gram negative rods, *Salmonellae* are relatively resistant to various environmental factors. The survival of *Salmonellae* for prolonged period of time in foods stored at freezer and ambient temperature is well documented. The viability of *Salmonellae* in dry foods stored at ≥25^{o}C decreases with increased storage temperature and with increased moisture content (WHO, 1988; D'Aoust, 1997). Although the low a_w in dry foods is not conducive to bacterial growth, the condition does promote survival of *Salmonellae* for prolonged period of storage at ambient and elevated temperature. The resistance of salmonellae to heating depends on medium in which they are kept; greater heat resistance observed in *Salmonellae* grown in nutritionally rich media than of *Salmonellae* grown in minimal media (Seifert, 1996; D'Aoust, 1997).

Salmonella is sensitive to heat and will not survive temperature above 70^{o}C. They are killed rapidly by autoclaving at 120^{o}C. *Salmonella* have been shown to be resistant to drying events for years, especially in dried feces, dusts, and other dry materials such as feeds and foods. Prolonged survival in water and soil has been described. The usual disinfectants (creolin3%, chalk milk 5%, caustic soda 2%) inactivate the pathogen in a few minutes. *Salmonella* are quit sensitive to gamma and beta radiation (Molla, 2004).

2.4. Epidemiology

The epidemiology of *Salmonella* is complex. *Salmonella* is one of the leading causes of bacterial food borne diseases in industrialized as well as developing countries even though the incidence seems to vary between countries and geographical areas depending on climate, population density, land use, farming practice, food harvesting and processing technologies, and consumer habits (Radostitis *et al.*, 1994). The wide variation in the national prevalence of *Salmonella* likely arise from limited scope of studies and lack of coordinated epidemiological surveillance systems, under reporting of cases and the presence of other diseases considered to be of high priority (Radostits *et al*., 1994; D'Aoust, 2000; 2001; Molla *et al.*, 2003a; Teklu, 2008).

The last few decades have witnessed marked increase in the incidence of human *Salmonella* infections. Several factors, including tourist travel, international movement of food and food ingredients, animal feed trade and the importation of infected animal as replacement stocks contribute to the national succession of *Salmonella* serotypes in human population and in the food chain. The prominence of agricultural products as major reservoirs of *Salmonella* species arises from the ubiquity of the microorganism in the natural environment; the intense on farm husbandry practices that favor the spread of *Salmonella* among reread animals, the use of untreated sludge to fertilize agricultural land and rendering of animal offals in to frequently contaminates feed proteins. Generally the complexity of epidemiology and many routes by which the organism can be transmitted presents a major challenge to all involved in animal production and controlling *Salmonella* (D'Aoust, 2001; MØlbak *et al*., 2006).

2.4.1. Distribution

Salmonella are widely distributed in nature and commonly found in farm effluents, human sewages, and any material subjected to fecal contamination. The main reservoirs of these bacteria is the intestinal tract of man and warm and cold- blooded animals (Jakabi *et al*., 1999), except fish, mollusks and crustaceans, which may get contaminated

after being fished. Among warm-blooded animals, cattle, chicken, geese, turkeys and ducks are the most important reservoirs. Domestic animals such as dogs, cats, turtles and birds may be carriers, and may pose great risk, mainly to kids (Girmay, 2013).

The distribution and importance of serovars involved in salmonellosis in human and animals vary over time for usually quite unknown reasons. Some serovars maintain their dominant role over many years, others, emerge, or reemerge or decrease over time. Therefore, a future of *Salmonella* infections in all species is a continual fluctuation in the proportion of the serovars involved. It is common for the serovars to be introduced to a country and to establish in one or more species, possibly as the predominant serovars and then to decline without any apparent reason or without intervention of public health or veterinary authorities (Alemu, 2007).

Salmonellosis has been recognized in all countries but appear to be most prevalent in areas of intensive animal husbandry. Approximately 2500 different *Salmonella* serovars have been described and number increase annually as new serovars are recognized (Wray and Davis, 2003).

The global distribution of the 15 most frequently identified serovars of *Salmonella* isolated from humans from 2001 to 2007 in laboratories from 37 countries that participated in World Health Organization Global Foodborne Infections Network and demonstrated serotyping proficiency in the Global Foodborne Infections Network External Quality Assurance System revealed; in all regions throughout the study period, with the exception of the Oceania and North American regions, *Salmonella* serovars Enteritidis and Typhimurium ranked as the most common and second most common serovar, respectively. In the North American and Oceania (Australia and New Zealand) regions, *Salmonella* serovar Typhimurium was the most common serovar reported, and *Salmonella* serovar Enteritidis was the second most common serovar. *Salmonella* serovars Newport (mainly observed in Latin and North American and European countries), Infantis (dominating in all regions), Virchow (mainly observed in Asian, European, and Oceanic countries), Hadar (profound in European countries), and Agona

(intense in Latin and North American and European countries) were isolated. There were large differences in the most commonly isolated serovars between regions, but lesser differences between countries within the same region. The results also highlight the complexity of the global epidemiology of *Salmonella* and the need and importance for improving monitoring data of those serovars of highest epidemiologic importance (Hendriksen *et al*., 2011).

2.4.2. Host range

Salmonellae have a wide range of domestic and wild animal hosts. The majority of serovars of *Salmonella enterica* show no host adaptation (Libby *et al*., 2004). However, some serovars appear to show degree of host adaptation and primarily infect one animal species. For example the host restricted serovars are *S.* Abortusequi (horse), *S.* Gallinarum and *S.* Pullorum (poultry), *S.* Typhisuis (swine) and *S.* Abortusovis (sheep). Some host adapted serovars are most prevalent in one animal species, but are also able to cause severe illness in the limited number of the hosts. These serovars include *S.* Choleraesuis (predominantly in swine and human), *S.* Dublin (predominantly in cattle and human, although occasionally the outbreak of disease caused by this serovar occur in sheep) (Wray and Davies, 2003). In contrast the non specific serovars are not restricted to a single host but able to colonize and on occasion cause severe illness or gastroenteritis in a wide range of animal species. For example *S.* Typhimurium and *S.* Enteritidis are frequently isolated from variety of vertebrates with and without clinical diseases and may be considered the least host adapted serovars. Typically, host adapted serovars cause severe systemic diseases in adult as well as young hosts, where as the unadapted serovars are associated with the enteric diseases primarily in young hosts (Libby *et al.*, 2004).

Today more than 2579 different serovars are known to man but only a limited number of approximately 50 serovares are predominantly found in domestic animals. The primary reservoir for majority of the remaining serovars remains obscure. Several factors complicated a clear picture of the true link between the serovars and the animal reservoirs; the production system (intensive/extensive), irrigation (manure) and

contamination of food sources (cross contamination). In addition only limited number of countries established a systemic integrated laboratory-based surveillance system, which includes data from both food and animals. Despite these factors some serovars appear to be more frequently associated with certain animal species and production system than others (Girmay, 2013).

2.4.3. Source of infection and modes of transmission

Salmonellae are mainly transmitted by fecal- oral route. Infected animals, which they excrete and infect other animals directly or indirectly by contamination of the environment; primarily the feed and water supplies are the sources of the organisms. Feed is a major potential route by which the new infections may be introduced in to a herd or flock. Many new serovars have been introduced in to countries in imported feed ingredients. Hay and basic cereal ingredients such as barely or maize can be contaminated during storage, by wild animals, especially rodents or birds but not considered particularly susceptible to *Salmonella*. Carnivores are also infected through meat, eggs and other animal products that are not thoroughly washed. Cats sometimes acquire *Salmonella* Typhimurium after feeding on infected bird or spending time near bird feeders (Alemu, 2007).

Contaminated drinking water may facilitate the rapid spread of *Salmonella* among farm animals, which often defecate in their drinking water. Contamination may occasionally occur in the water storage tanks in a building, from wild feces or even from carcasses. Animals may be infected either by drinking such water or from contamination of pasture when flooding occurs. Most infections are introduced in to *Salmonella* free herd by purchase of infected animals for intensive rearing or adult animals for replacement. Purchase animals may have acquired infection on their home farm premises, on transit or dealer's premises (Teklu, 2008).

Vertical transmission occurs in birds with contamination of vitelline membranes, albumin and possibly the yolk of eggs. *Salmonella* silently infect the ovaries of

apparently healthy hens and contaminate the eggs before the shells are formed (Girmay, 2013).

People are often infected when they eat contaminated foods of animal origin such as meat, milk or eggs. Ingesting organisms in animal feces can also infect them, either directly or in contaminated food or water. Directly transmitted human infections are most often acquired from feces of reptiles, chicks, and ducklings. Live stock, dogs, cats, adult poultry and cage birds can also be involved (OIE, 2005).

2.4.4. Pathogenesis

Many of the existing serovar of *Salmonella enterica* have unique characteristics regarding their host specificity and pathogenesis. Transmission of *Salmonella* is usually by the fecal- oral route but infection via mucous membranes of conjunctiva and upper respiratory tract is suspected (Quinn *et al.*, 1994).

Colonization of the distal small intestine and the colon is a necessary first step in the pathogenesis of enteric salmonellosis. Indigenous fusiform bacteria that lie in the mucous infesting the epithelium of the large intestine normally inhibit the growth of *Salmonella* by producing volatile organic acids. Following ingestion, the microorganism must survive exposure to the low PH of the stomach. Despite having a number of systems to resist acidity, only small percentage of ingested *Salmonella* survive exposure to low PH, usually can survive to about PH 3, and move on into small intestine (Libby *et al.*, 2004).

The normal flora blocks access to attachment sites needed by the pathogens. Factors which disrupt the normal colonic flora, such as antibiotic therapy, diet, and water, deprivation, greatly increase the host's susceptibility to enteric and septicemic salmonellosis. Reduced peristalsis also enhances colonization by *Salmonella* because it allows temporary overgrowth to occur, especially in the small intestine. Peristalsis is stimulated by an active indigenous microflora, suppression of which increases the host's susceptibility to colonization (Venter *et al*., 1994).

In the small intestine, bacteria move across the intestinal epithelial cell and reach the M cells, thus penetrating in the Peyer's patches. The M cells are specialized epithelial cells overlying Peyer's patches that have probably originated from intestinal epithelial cells and small pockets in the mucosal surface. After contact with M cells, the infectious bacteria are rapidly internalized. *Salmonella* have evolved intricate measures to invade host cells following the epithelial attachment. Upon its interaction with host cells, Type III protein secretion systems (T3SS) facilitate endothelial uptake and invasion (Denise *et al*., 2004).

The type III secretion system (T3SS) proteins encoded by two *Salmonella* Pathogenicity Islands (SPIs) which are associated with the pathogenicity at molecular level. The SPI can be located on the chromosome or on a plasmid and characterized by a base composition different from the core genome and are often associated with tRNA genes and mobile genetic elements, like islet (IS) elements, transposons or phage genes (Schmidt and Hensel, 2004). By now, 15 SPIs have been identified; two of these, SPI-1 and SPI-2 encode T3SS**.** The T3SS1 encoded by SPI-1 contains invasion genes; while T3SS2 encoded SPI-2 is responsible for intracellular pathogenesis and has a crucial role for systemic *S. enterica* infection (Kaur and Jain, 2011).

Salmonella can also actively invade both phagocytic and non-phagocytic cells using a type III secretion system (T3SS), T3SS1. T3SS1-mediated invasion by *Salmonella* is a highly specific process that depends on the tightly regulated expression of a number of bacterial factors. The biological function of T3SS1 is the translocation of proteins from the bacterial cytoplasm into the host cell, thus functioning as ‘molecular syringes’. The assembly of the SPI-1 T3SS1 to molecular syringe appears to be built from the base up. An assembly model starts with the assembly of the inner ring structure, which spans the cell membrane and is assembled from PrgH and PrgK protein subunits. Next, the cytoplasmic export machinery, which is composed of the InvA, InvC, SpaP, SpaQ, SpaR, and SpaS proteins, is assembled. Also, the outer ring structure, composed of InvG and InvH, is assembled in the outer membrane and connected to the inner ring structure and is stabilized with the aid of the regulatory protein InvJ. The completed base structure allows

for the assembly of the needle and inner rod structures, which are made up of PrgJ and PrgI subunits, respectively (Antonio and Steele- Mortimer, 2009).

The completed SPI-1 T3SS allows for effector proteins to be translocated from the bacterial cytoplasm to the host cell. In the bacterial cytoplasm, chaperone molecules bind to the effector proteins and accompany the molecule to the export machinery of the T3SS. In a remarkably co-ordinated process a small group of effector proteins (SipA, sipB, SipC, SOP A, SopB/SigD, SopD, SopE2 and SptP) induce dramatic rearrangement of the actin cytoskeleton resulting in massive localized membrane ruffles and rapid internalization of the bacteria (McGhie *et al*., 2009).

Once *Salmonella* is internalized, the microorganism resides in the membrane bound vacuole/*Salmonella*-containing vacuole (SCV). As the SCV matures, it migrates from the luminal border of the cell to the basal membrane where the *Salmonella* interact with and enter into the macrophages associated with Peyer's patches in the submucosal space. The formation of SCV occurs separately from the normal endocytic processing pathways present in host cells. Further, it also acquires some of the endosomal markers involved in intracellular processing; though, it does not fuse with lysosomal compartments. More recently, SopB was found to manipulate the SCV surface charge resulting in the inhibition of SCV and lysosome fusion. This separation helps, *Salmonella* to avoid being killed by the normal phago-lysosomal processing pathways. The SCVs are important for *Salmonella* survival and transport in epithelial cells and play a key role in the survival of the bacterium within phagocytic cells such as macrophages during invasive infections. Therefore, the ability to survive and proliferate in SCV is very important for virulence of *Salmonella* (Kaur and Jain, 2011).

Invasive *Salmonella* infections are associated with T3SS encoded on SPI-2. The SPI-2 T3SS genes are only expressed inside the host cell containing SCV. A number of environmental conditions have been associated with the induction of the expression of SPI-2 T3SS genes through the OmpR-EnvZ regulatory system, including low osmolarity, low levels of certain nutrients, and acidification of the SCV. The activated SPI-2 T3SS

facilitates the transfer of effector proteins from *Salmonella* across the SCV membrane to interact with targets in the host cells. Many SPI-2 T3SS effector proteins including, SifA, SifB, SseJ, SseF, SseG, PipB and SopD2 interact with microtubule bundles and their associated motor proteins and are involved in the formation of *Salmonella*-induced filaments (SIF) that extend from SCV. The formation of SIF takes place as the result of the fusion of SCV with other vesicles in the cell. The major functions of SIF are not fully understood; however, it is likely that SIF are important for pathogenesis and may play a role in intracellular replication of *Salmonella* because their formation often coincides with replication of the microorganisms. SpiC is translocated into the cytosol of host macrophages, where it interacts with the endomembrane system and interferes with normal secretory pathways of the host. This disruption likely protects organisms from bactericidal compounds, including reactive oxygen and reactive nitrogen molecules that are able to kill bacteria (Kaur and Jain, 2011; Antonio and Steele- Mortimer, 2009).

Systemic infections are severe manifestations of salmonellosis. To facilitate systemic infection, intracellular *Salmonella* present in immune cells such as macrophages and dendritic cells (DC) may be carried from the intestinal tract to other areas of the body. Dendritic cells are important migratory phagocytes that are widely distributed throughout the body in lymphoid and non-lymphoid tissues. The ability of DC to migrate throughout the body potentially facilitates the spread of *Salmonella* to various parts of the body. While in DC, the *Salmonella* do not appear to replicate but remain viable, possibly in a small colony variant state with reduced metabolic activity and increased persistence. Genes encoded on SPI-2 T3SS appear to suppress antigen presentation by DC, which limits a robust immune response by the infected cells. A proteomics analysis of *S.* Typhi grown in low pH, low magnesium, minimal media (MgM or LPM) was reported. MgM is designed to approximate the phagosome of infected macrophages and is known to induce expression of SPI-2 virulence genes and other genes related to virulence and intra macrophage survival. The combination of lowered metabolic activity and immunosuppression contributes to the persistence of *Salmonella* within host cells. When macrophages or DC enter the organ systems, the *Salmonella* can spread to adjacent cells

and trigger apoptosis, which leads to increased pathology among the infected cells (Tierrez and Garcia-del Portillo, 2005).

One of the major clinical features of salmonellosis is diarrhea, which is caused by SPI-1 T3SS translocated proteins. The SopB protein appears to play an important role in the activation of secretory pathways, the attraction of neutrophils to the sites of infection (thereby increasing inflammation), and an alteration of ion balances within cell. Additionally, SopB which probably influences the ion balance in cells through the antagonism of chloride channels in the infected cells. The alteration of ion balances within the cells can lead to fluid secretion into the intestinal tract and subsequent diarrhea. Other proteins such as SipA, SopA, SopD, and SopE2 may also play a role in *Salmonella*-associated gastroenteritis (Antonio and Steele- Mortimer, 2009; Kaur and Jain, 2011).

2.4.5. Carrier state

Salmonellae are facultative intracellular organisms that survive in the phagolysome of macrophages and can evade the bacterial effect of antibody and complement (Radostits *et al*., 1994). Carrier animals harbor organism in their mesenteric lymph nodes, gut associated lymphoid tissue, macrophages in the lamina propria of the intestine, and gall bladder (Venter *et al*., 1994). *Salmonella* Dublin localizes in gall bladder and spleen of cattle, and carriers may shed the organism for years (Wigley *et al*., 2001).

When animal is infected with *Salmonella* it may become a clinical case or inactive carrier, passing an organism constantly or intermittently in feces. It may also become a latent carrier with infection persisting in lymph nodes or tonsils but no *Salmonella* in feces or a passive carrier which is constantly picking up infection from pasture or calf pen floor but the infection disappears when it is removed from the environment. These animals probably multiply *Salmonella* without becoming permanent carriers. The importance of latent carrier is that they can become active carrier or even clinical cases under stress, especially at calving time. Thus, the persistence infection in animals and in

the environment is important epidemiological features of salmonellosis. In sheep and cattle the carrier state may persist for as long as 10 weeks, in horses up to 4 months (Radostits *et al*., 1994; Jones *et al*., 2004).

2.5. Clinical features

2.5.1. Salmonella infections in humans

Salmonellosis is an infection with *Salmonella* bacteria transmitted through feco-oral route. Most people infected with *Salmonella* develop diarrhea, fever, vomiting, and abdominal cramps 8 to 72 hours after infection. In most cases, the illness lasts 4 to 7 days and most people recover without treatment. However, in some persons the diarrhea may be so severe that the patient becomes dangerously dehydrated and the *Salmonella* infection may spread from the intestines to the bloodstream, and then to other body sites such as the blood stream, the meninges, bone or joint spaces and can cause death unless treated. *Salmonellae* produce three main types of disease in humans, but mixed forms are frequent. These are enteric fever (typhoid), septicemia and enterocolitis and rarely chronic carriers may develop gall bladder stone (Boyle *et al*., 2007; Ibrahim, 2013).

Some patients may harbor *Salmonella* species in stool or urine for periods of 1 year or longer but remain asymptomatic. Approximately 3% of patients with typhoid fever and 0.2–0.6% of persons with non-typhoid *Salmonella* gastroenteritis will have positive stool cultures for more than 1 year where the organism present in the gallbladder, biliary tract, or rarely the intestine or urinary tract. *Salmonella enterica* serovar Typhi can establish a chronic, asymptomatic infection of the human gallbladder, suggesting that this bacterium utilizes novel mechanisms to mediate enhanced colonization and persistence in a bile-rich environment by forming a biofilm. Bacteria reaching the gallbladder can induce an active local infection (cholecystitis) or exist asymptomatically in a chronic carrier state. The chronic typhoid carrier state can occur following symptomatic or subclinical infections of *Salmonella* Typhi (Fentabil, 2011).

2.5.2. Salmonellosis in Cattle

In cattle the enteric and septicemic syndrome of salmonellosis are common than the abortion syndrome. One or more of the syndromes may occur simultaneously in an outbreak of salmonellosis in a heard, or even in a single affected animal. *Salmonella* Dublin is more often the cause of septicemia than *Salmonella* Typhimurium. Calves are more likely than adult animals to suffer from septicemic infections and may develop peracute, acute or chronic salmonellosis. The peracute disease is usually a septicemic condition, it is often fatal and calves may die suddenly without premonitory sign. Some, however, develop enteritis and diarrhea in addition to septicemia. When the course of septicemia is protracted, sign of hepatitis, pneumonia, meningoencephalitis, polyarthritis and osteomyelitis may develop (Alemu, 2007; Luciana *et al*., 2012).

Acute enteric salmonellosis is the most common syndrome of salmonellosis encountered in calves. Following an incubation period, which may vary from one to five days, calves develop high fever, in appetence, lethargy, diarhoea, and frequently a serous nasal discharge followed by a slight cough and scouring may only occur terminally. The feces of affected calves have foul smelling, putty-like consistency, and contained large amount of mucous, sloughed mucosa and flecks of blood. At this time body temperature may be normal or subnormal. The mortality rates are 5 to 10% and may reach as high as 75% in purchased calves (Venter *et al*., 1994; Wray and Davies, 2000).

Chronic salmonellosis in calves is characterized by unthriftiness, long and scruffy hair coats and stunting. Diarrhea is not always present and sign of chronic pneumonia with persistent coughing may occur. The calves that survive either peracute, acute or chronic disease may develop pneumonia, meningoencephalitis, purulent polyarthritis, and osteomyelitis of the vertebrae and bones of distal parts of limbs, resulting in lameness, paresis or even almost complete paraplegia. Dry gangrene of the skin of the lower limbs and the tips of ear and tail, resembling ergotism, is rarely encountered (Alemu, 2007).

Adult cattle generally contract either acute or subacute enteric salmonellosis, and pregnant animals may abort. During the early stage of acute enteric disease, severely affected animals show fever, depression, inappetance, and a drop in milk yield. The signs are followed by diarrhea, which has foul smelling, the feces being mucoid and usually containing clots of blood, and shreds of necrotic intestinal mucosa. Signs of colic, congested mucos membranes and dehydration may be evident. Most of the signs are associated with endotoxemia induced by the lipid, the lipid A component of lipopolysaccharide in the outer layer of gram negative bacterial cell wall. The acute disease lasts for about a week. The case fatality rate in adult animals with dysentery 50 percent, but may be higher. Complete recovery may take up to two months. Similar but less sever signs are present in animals suffering from subacute enteric salmonellosis, but most affected animals recover without treatment (Radostitis etal., 1994; Venter *et al.*, 1994).

Salmonella Dublin in particular, but also other serovars, may cause abortion in cow at any stage of pregnancy. Abortion may either precede the onset of dysentery or follow it within two to four weeks. Alternatively abortion may occur in cows that show no sign of ill health septicemia and/or placentitis being the cause of death of the fetus. Retention of the placenta occurs in approximately 70% of the cows that abort, but subsequent fertility is not affected (Venter *et al.,* 1994).

Both live and killed vaccines are available for prevention of salmonellosis in cattle. Calves may become infected with in few days after birth and peak mortality occurs between three and four weeks of age. Therefore, passive protection by vaccination of adult would appear to be the ideal way of protecting calves (Jones *et al*., 2004).

2.6. Culture, isolation and typing

Clinical signs and findings at post-mortem examination are not unique to salmonellosis and also a tentative diagnosis may be made this should be confirmed in diseased animals or at necropsy by isolation of the organism (Jones *et al.,* 2004). Numerous culture

protocols have been developed and modified to reliably recover and characterize *Salmonella* species from a broad range of sources. On farm hazard analysis critical protocol point (HACCP) approaches for improving food safety and animal health have resulted in attempt to optimize antemortem, postmortem and environmental specimen collection, processing, and culture for *Salmonella* detection (Maddox, 2003).

Emphasis has been given for *Salmonella* testing methods that provide results more rapidly, with sensitivity similar or greater than the conventional methods. These rapid methods should be robust and reliable and have specificity that minimizes false- positive results and at a cost that is not prohibitive. A wide range of alternative approaches have been under taken, for detection of *Salmonella* in food and many of these techniques are undergoing continued development, with similar to standard culture methods, but poor performance have often led modification and improvement to techniques (Blackburn, 1993; Maddox, 2003).

Recent emphasis on food safety issues and development of guidelines using *Salmonella* as an indicator for enteric contamination of meat and potential pathogen in animal products has led to efforts to standardize detection techniques (Maddox, 2003). Standard culture method for detection of food borne *Salmonella* serovars generally consists of pre-enrichment in on non selective liquid medium, enrichment in selective liquid medium, plating on differential agar, biochemical screening and serological confirmation of identity (ISO 6579, 2002).

A variety of enrichment technique and isolation media are available for cultivation of *Salmonellae.* They rely on promoting selective growth of *Salmonella*, whilst inhibiting the growth of contaminants, and identification on the basis of colony morphology and biochemical reaction. However, the choice of media depends up on environment from which the organism must be isolated and often depends on the subjective choice of bacteriologists who specialize in *Salmonella* isolation (Jones *et al*., 2004). Media bias often results in reduction in recover from media other than that used in his or her laboratory (Maddox, 2003).

2.6.1. Pre-enrichment in non selective liquid media

Salmonella may be present in small number in particular mass or volume of products and are often accompanied by considerably large number of other *Enterobacteriaceae* or other family it may be sublethally injured (ISO 6579, 2002). For example the number of *Salmonella* in feces from asmptomatic animals, environmental samples, animal feed and food is usually low (D'Aoust, 2001) and may not be detected by conventional culture methods (Wray and Davies, 2003). The study in 'ayib' (Ethiopian cottage cheese) reported that a higher recovery of *Salmonella* has been observed in all methods involving pre-enrichment procedures. Therefore a test proportion is initially inoculated in to nutritious non inhibitory pre-enrichment liquid medium to favor the small number of *Salmonella*, which may otherwise killed by the toxic effect of enrichment media, to multiply to detectable level or it may help to resuscitate *Salmonella* that have been stressed, sublethally injured arising from exposure to heat, freezing desiccation, preservatives, high osmotic pressure wide temperature fluctuation. Generally pre-enrichment media are nutritionally complex. Traditional pre-enrichment media include non fatty dry milk added with brilliant green dye for the pre-enrichment of cocoa and chocolate products, brilliant green water for milk powder, tripticase soy broth supplemented with potassium sulfite to neutralize spice-dependent bacteriostasis, nutrient broth, lactose milk and buffered peptone water (D'Aoust, 2001; ISO 6579, 2002).

2.6.2. Enrichment in selective media

Enrichment in selective liquid media may follow an initial enrichment in non –selective liquid media or it may be used as an initial step in specimens containing high number of competitive bacteria such as feces or ground meats to prevent over growth by coliforms that can readily out–compete the Salmonella (Maddox, 2003). In general a large number of liquid enrichment media are necessary to guarantee the isolation of majority of serovars. Enrichment media depends up on allowing *Salmonella* to grow, although some times in an inhibited manner, whilst suppressing other bacteria through chemicals, dyes, antimicrobials and enhanced incubation temperature. In common used are media

containing sodium selenite or tetrathionate and dyes such as brilliant green or malachite green to which *Salmonella* are relatively resistant (D'Aoust, 2001; ISO 6579, 2002).

2.6.3. Plating out and identification

Enrichment cultures are plated on selective agar medium for presumptive identification of *Salmonella* colonies on basis of discriminating biochemical reactions. Standard plating media include brilliant green agar (BGA), Bismuth sulfite agar (BSA), Xylose-lysine-deoxycholate (XLD), and Hektien enteric (Hek) agars that report on acid production from lactose and/or sucrose utilization through determinant colour changes in the media. *Salmonella* spp. that typically produce hydrogen sulfide appear as charcoal black colonies with or without black halo that produce metallic sheen under reflected light. The Rambach and SM-ID plating media produce discriminating colour reaction in the presence of isolates that are β-galactosidase positive and that produce acid from propylene glycol (Rambach) and Glucuronate (SM_ID). Plating media generally yield suspected colonies within 18-24 hrs incubation at 35-37^{O}C, except BSA, which may require 48hrs for development of presumptive *Salmonella* colonies. Comparative studies support the following ranking in decreasing order of effectiveness: BSA> BGS> BGA≥ Rambach= SM-ID> XLD>Hek (D'Aoust, 2001; ISO 6579, 2002).

2.6.4. Confirmation

For confirmation, at least five (typical or suspected) *Salmonella* colonies will be selected from every selective plating media. If the suspected colonies on each plate are fewer than five, all colonies will be selected. The selected colonies will be plated on surface of pre-dried nutrient agar plates, in a manner that will allow well isolated colonies to develop. Then the incubated plates will be incubated at 37^{O}C ± 1^{O}C for 24±3hrs. The pure culture on the nutrient agar will be used for biochemical and serological confirmation (ISO 6579, 2002).

Biochemical testing

Salmonella are chemoorganotrophic, with an ability to metabolize nutrients by the respiratory and fermentative pathways. The organisms catabolize D-glucose and other carbohydrates with production of acid and gas. *Salmonellae* are oxidase negative and catalase positive, grow on citrate as sole carbon source, generally produce hydrogen sulphide, decarboxylate lysine and ornithine, and do not hydrolyze urea (D'Aoust, 1997; Quinn *et al.,* 1999).

Colonies characteristic of *Salmonella* on the selective/indicator media are inoculated, singly into a triple sugar iron (TSI) agar slope and lysine decarboxylase broth. The typical reaction for *Salmonella* on TSI agar is a red slant (alkaline) slant, yellow (acid) butt and super imposed hydrogen sulphide (H_2S) black color production. When lactose positive *Salmonellae* are isolated, TSI slant is yellow. Thus, preliminary confirmation of *Salmonellae* cultures shall not be based on the result of TSI agar only. The test for lysine decarboxylation is positive. However, *S.* Choleraesuis donot produce H_2S although *S.* Choleraesuis biotype Kunzendorf is H_2S positive (ISO 6579, 2002).

Salmonella generally are β-galactosidase, vogus-proskaur and indole test negative. However *Salmonella* species as a biochemically homogenous group of microorgamsms is rapidly diminishing. The situation will likely lead to reassessment of the diagnostic value of biochemical traits and to their likely replacement with molecular technologies targeted at identification of stable genetic loci and/ or their products that are unique to the genus *Salmonella* (D'Aoust, 1997).

2.6.5. Typing

Typing method is any method that can be used to differentiate bacteria beyond species level. Outbreak investigation and tracing of zoonotic bacteria among live stock and from livestock via food to man can be performed by the use of bacterial typing methods. These

methods can subdivide a bacterial species in to individual colonial lines, group of bacteria produced by continuous division of cells from same ancestor (Olsen *et al.*, 1993).

There are phenotypic and genotypic typing methods. Originally phenotypic typing methods such as serotyping, phage typing and biotyping, are the only methods applied. These traditional methods still play a very important role in tracing of bacteria, and sporadic case of salmonellosis, for example, will only be grouped to form a tentative out break if the isolates show identical serotyping, and where applicable, identical phage typing results (Olsen *et al.*, 1993).

Serotyping

Serotyping is based on O and H-antinegens using slide agglutination test. Most serotypes exhibit diphasic flegellar antigen expression by alternatively expressing two genes, fliC(phase 1) fliB which encodes flagellins of different antigenecity. *Salmonella* serotyping methods recognize 63 distinct phase 1 flagellar antigenic factors and 37 phase two flagellar antigenic factors although the latter are not always present (Mortimer *et al.*, 2004).

Bacterial growth for serotyping should be taken from a triple iron (TSI) agar slant from nutrient agar as culture as culture from selective media is often unsuitable for serotyping. Then a loopful of culture of the *Salmonella* to be serotyped should be suspended in a drop of saline on a microscope slide and examined for autoagglutination. This can occur with strains and will invalidate the serotyping. Smooth rough dissociation occurs after sub culture and most frequently from media containing carbohydrates. Smooth *Salmonella* to be serotyped is emulsified in a drop of 0.85 % saline microscope slide. A drop of antisera is added to and mixed well with *Salmonella* suspension. The slide is rocked gently for about 30 seconds and the antigen-antibody mixure examined for agglutination. The *Salmonella* is first tested against antisera to O antine them H antigen (Quinn *et al*., 1999).

Phage typing

Some serovars have several different phenotypes, and their identification can be important in epidemiological investigation. Phage typing scheme for *Salmonella enterica* serovars are based on pattern of lysis produced by distinct phages isolated from variety of sources. Pure culture of bacteria is flooded on to plates and suspension of typing phage is spotted on to plates. Strains that are susceptible to suspension of typing phages are allocated to sama phage type. As this typing method is cheap and labor in expensive, it is normally the second method to apply in the study of *Salmonella* epidemiology, and phage typing scheme have been developed for many important salmonella serovars. Phage typing is the principal method of typing in *S.* Enteritidis and *S.* Typhimurium and has been used to subdivide isolates within serovars Typhi, Typhimurium, Enteritidis, Virchow, Hadar and Heidelberg. Although phage typing is essential for sub division of *Salmonella* serovars, the method can prove inadequate for serovars in which a small number of phage types predominate (Olsen *et al*., 1993; Quinn *et al.,* 1999; Heuzenroeder, *et al*., 2004).

Antibiogram typing

Resistant to antimicrobial agent is considered to be relatively unstable, because majority of bacterial resistance factors are carried on plasmid that are often transferable between strains which may be dependent on selection pressure to be stably maintained. Antibiograms are rarely used as the only typing methods. The epidemiological significance is secondary to the implication for therapy and control. However the result of these investigations can readily be used for epidemiological purpose. The spread of multiple antimicrobial resistant strains among livestock is often traced using the antibiogram as typing methods, preferably combined with other typing methods (Alemu, 2007).

Molecular and genetic typing

These methods involve plasmid profiling, plasmid restriction analysis, restriction analysis of the full genome and PCR-based typing. Molecular typing is used when conventional methods fail to give sufficient determination between isolates. Bacterial genomes can be compared by electrophoretic separation of DNA-fragments generated in Vitro by digestion with restriction endonuclase enzymes. Pulsed field gel electrophoresis has been in use for some considerable time and is accepted gold standard adopted by organizations such as CDC in Atlanta. Plasmid profiling and plasmid restriction analysis has been extensively used for typing of salmonella, often to fine tune conclusions based on phage typing result (Molla, 2004).

2.7. Treatment

Current recommendations are to most patients with uncomplicated *Salmonella* infection with supportive therapy and no antimicrobial agents; however, many receive empiric therapy (or take self-treatment) without a stool culture. Antimicrobial therapy is recommended for gastroenteritis caused by *Salmonella* species in people at increased risk of invasive disease (infants aged <3 months, older adults aged ≥60 years, the debilitated or immune suppressed) and patients with continued high fever or manifestations of extraintestinal infection. Fluoroquinolones are often employed for empiric treatment; azithromycin and rifaximin are also commonly used to treat travelers' diarrhea. Resistance to antimicrobial agents varies by serotype and geographic region. Resistance to older antimicrobial agents (chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) has been present for many years, and resistance to both fluoroquinolones and third-generation cephalosporins has been reported (CDC, 2013).

It is also important to search for endovascular abnormalities by using imaging techniques in older patients with or without evidence of atherosclerosis. Although there is no consensus on optimum duration for post operative antibiotic therapy for endovascular infections caused by salmonella, most investigators still recommend a minimum of six

weeks. The duration of therapy for other extra intestinal infections should be considered based on the site of infections. In general, 10 to 14 days for bacteremia (Chiu *et al*., 2004).

2.8. Vaccination

Vaccines are the most powerful biologicals which have modulated the economic, social and cultural life of human beings. Certain diseases have haunted humanity for centuries but are now extinct due to vaccines. On the other hand, some diseases such as salmonellosis, that was uncontrollable in the past, still cause pandemics. Different investigations and practical experiences have provided evidence that vaccination play a role in the prevention and control of animal salmonellosis. Three major types of vaccines are being used to control salmonellosis: killed bacteria, subunit vaccines and live attenuated vaccines (Singh, 2009).

For inactivating *Salmonella* in killed vaccine, agents such as heat, ß-propiolactone (BPL), glutaraldehyde and formaldehyde have been tried to preserve the antigenicity and increase the efficacy of vaccines with addition of potent adjuvants such as Chrome alum, alhydrogel, mineral oil, potash alum, Freund's incompleteand Freund's complete adjuvant (FCA). Use of various adjuvants which enhanced not only immunogenicity but also increased length of the protection periods. Killed vaccines are commercially available for *S.* Typhimurium, *S.* Abortusequi, *S.* Dublin, *S.* Virchow, *S.* Gallinarum and *S.* Enteritidis (Singh, 2009).

Live vaccines are prepared by cultivating organisms under conditions that disable their virulence properties. Live vaccines for *Salmonella* have also been used in a number of countries; these include 9R and 9S strains of *S. enteric* ssp. *enterica* serovar Gallinarum (*S.* Gallinarum) for control of fowl typhoid and HWS51 *S.* Dublin infections. Live vaccines have also been prepared from genetically defective (auxotrophic) mutants, selected for their requirement for metabolites that are absent or present in insufficient quantity in the immunized animals. For example, vaccine of purine-requiring *S.* Dublin,

S. Choleraesuis and S. Typhimurium and aromatic amino acid requiring *S.* Typhimurium and *S.* Dublin are available commericially (Woodward *et al*., 2002).

Comparative analysis of live and killed vaccines revealed that killed vaccines are usually less effective for three reasons. Firstly, they only contain surface antigens that give an incomplete protective antibody response; secondly, they fail to elicit cell-mediated immune response, which is important for long-term protection from salmonellosis and finally; they fail to elicit production of secretory immunoglobulin (sIgA) response critical for protection of mucosal surfaces from colonization with the pathogen. Attempts to overcome all three problems, by culturing vaccine candidates under iron limiting microaeropilic conditions, through use of adjuvant to induce cell mediated immunity (CMI) and mucosal immunity (sIgA) gave only partial success. Oral immunization with live or inactivated vaccines stimulates local intestinal immunity and has been shown to be effective in decreasing the excretion of *Salmonella* by infected animals. Cross protection against other *Salmonella* serotypes is not observed following immunization with inactivated vaccines. However, an oral administered, live vaccine against *S.* Typhimurium has been shown to protect calves against challenge by *S.* Dublin. Killed vaccines, whilst not very effective, are still the best for use where the disease is eradicated and are the preferred choice for eradication of an endemic strain from a herd or when dealing with an outbreak of salmonellosis (Singh, 2009).

Sub unit vaccines are prepared by taking fragments from micro-organisms rather than introducing an inactivated or attenuated micro-organism to an immune system (which would constitute a "whole-agent" vaccine). Common sub-cellular components of *Salmonella* used for development of vaccines are: outer membrane proteins (OMPs), porins, toxins and ribosomal fractions. Such vaccines tried in different animals had variable success. OMP of *S.* Gallinarum adjuvanted with mineral oil caused 100% clearance of challenge strain of *S.* Gallinarum in birds vaccinated with 400 μg OMP/bird. The immune response of OMPs from *S.* Heidelberg and *S.* Gallinarum could be improved through lipid-conjugation with immunostimulating complex (ISCOM) and bacterin, respectively. Researches in India found ribosomal fractions and non-ribosomal proteins

(non-denatured bacterial cell envelops) of *S.* Typhimurium to be a potential vaccine candidate in a rabbit model (Charles *et al*., 2000).

Most of the subunit vaccines failed to afford significant protection either in field or in experimental models except a toxoid vaccine made from *S.* Weltevreden toxins which provided 100% protection in a mice model, there is hope for development of a broad spectrum *Salmonella* vaccine lies with sub-component and cytotoxin-I toxoid vaccines (Lee *et al*., 2000).

2.9. Control of salmonellosis.

Control is important to reduce both public health risk and economic losses. Control measures could be directed against the host, the agent or the environment. Measures directed toward the host include, increasing the resistance of animals in risk group by immunization and appropriate feeding to maintain intestinal flora in a balanced state. Separation of age groups and prevention of contact with other infected carrier animals lessen the risk of exposure; while isolation of diseased animals prevents the spread of the infection and contamination of the environment. The use of vaccines in the control of salmonellosis has been considered to be valuable only as an auxiliary provided that *Salmonella* population be restrained to reasonable level. Both live attenuated vaccines produced from rough strains and bactrins precipitated on aluminum hydroxide are commercially available (Radostits *et al*, 1994; Venter *et al*., 1994).

Treatment, isolation or elimination of animals suffering from salmonellosis, as well as disinfection of sheds and stables, should be implemented to prevent environmental contamination. *Salmonella* organisms are destroyed by most disinfectants that are effective against vegetative bacteria such as phenol, chlorine or iodine based compounds. Feeding utensils, equipments and transport vehicles should also be cleaned and disinfected (Venter *et al.,* 1994).

Measures aimed at environment include, institution and maintenance of hygiene and good animal husbandry practices such as regular removal of feces, keeping feed store rodent free, the use of separate milk pail for each calf, which are then thoroughly washed and disinfected after use, provision of suitable housing and prevention of overcrowding (Radostits *et al*, 1994).

2.10. Antimicrobial resistance

Resistance of *Salmonella* to commonly used antimicrobials is increasing in both veterinary and public health sectors and has emerged as global problem. Conventional antimicrobial agents, such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, had been the drug of choice in the treatment of salmonellosis before the 1980s. Recently antimicrobial resistance to streptomycin, ampicillin or combination sulfonamides is increasingly detected (Izzo *et al.*, 2011). Extended- spectrum cephalosporins and flouroquinolones have been suggested as appropriate alternative agents in the treatment of infections caused by such multidrug resistant *Salmonella* serotypes; however resistance to both fluoroquinolones and third-generation cephalosporins has been reported (CDC, 2013).

The increasing proportion of single and multiple antimicrobial-resistant *Salmonella* strains isolated from human salmonellosis cases has been associated with the widespread use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses. A considerable number of antimicrobials commonly used in the treatment of salmonellosis and other bacterial infections of humans are also used in veterinary practices. This may present a public health risk by the transfer of resistant *Salmonella* and other zoonotic bacterial pathogens or the resistant genes from food animals to humans through consumption of contaminated food and food products (Molla *et al*., 2003b; Zelalem *et al*., 2011). In addition, pet animals such as frogs and turtles and their water environment were shown to carry multidrug resistance *Salmonella* strains, which subsequently cause infections in humans. Therefore, to curb the resistance problem in

Salmonella, it has been suggested that inappropriate use of antimicrobial agents in food animal should be prohibited (Chiu *et al*., 2004).

2.11. Zoonotic and economic importance

Salmonella are zoonotic pathogens that cause food-borne illness and that are widely disseminated in nature. National and global epidemiological registries continue to highlight the importance of *Salmonella* spp. as one of the leading cause of food borne bacterial zoonotic diseases in humans. An expected number of 93.8 million human cases of gastroenteritis caused by *Salmonella* occur annually throughout the world of which 80.3 million cases are estimated to be foodborne and of which 155,000 infections result in death (Karoline, 2012). The cumulative impact of *Salmonella* infections on the economy are due to loss of work, medical care and loss of life, pain and suffering and loss of leisure time (Scharff, 2012). The cost of food safety regulatory programs and cost to the food industry for product recalls and plant closure due to food borne salmonellossis out break would increase the size of estimated lose. Moreover, the most serious risk is that the transmitted bacteria will have acquired resistance to specific antibiotics because the animal from which they originated has been treated with the particular antibiotics repeatedly over long period (Teklu, 2008). Moreover, various clinical forms of *Salmonella* such as gastroenteritis, bacteremia and other systemic abnormalities can occur in veterinarian working with *Salmonella*-infected animals. As such, ill and/or hospitalized horses and cattle must be considered as potential source of *Salmonella* for veterinary clinicians and students. In one study *Salmonella* could be isolated from 5.5% of hospital horses (http://www.vetmed.wisc.edu/pbs/zoonoses/gik9fel/salmonella.html).

The gross marginal loses losses arose from both direct losses such as reduced milk yield, dead animals, treatment costs and abortions as well as indirect losses such as reduced income from sold heifers and calves, and lower milk yield of replacement animals (Neilsen *et al.,* 2013).

3. MATERIALS AND METHODS

3.1. Description of the study site and study population

3.1.1. Study site

A cross sectional study was conducted in apparently healthy slaughtered cattle and lactating cows during December 2013 to June 2014 to estimate the prevalence and distribution of *Salmonella*, assess the antimicrobial resistance pattern and find out some of the risk factors of milk and carcass contamination in central Ethiopia. Five towns were purposively selected as study site representing central Ethiopia for sampling; Bishoftu, Adama and Addis Ababa from Shewa and Asella from Arsi zones of Oromia Regional state (the map of the area is attached in figure 2 of this paper).

Bishoftu

Bishoftu: is located in Oromia National Regional State about 45 km South-east of Addis Ababa, just on the escarpment of the Great Rift Valley and the geography of the area is marked by creator lakes. It is found at 9^0N latitude and 40^0E longitude and at an altitude of 1850 meters above sea level in the central high lands of Ethiopia. It has a human population of about 99,928. It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September (of which 84% of rain is expected) and a short rainy season from March to May with an average annual rainfall of 800 millimeters. The mean annual minimum and maximum temperatures are 12.3^0C and 27.7^0C, respectively, with an overall average of 18.7^0C. The highest temperatures are recorded in May and the mean relative humidity is 61.3%. Bishoftu is the center of Ada'a Woreda and it has a total land area of about 1610.56 Km^2 and is divided in to three agro-ecological zones namely midland (94%) highland (3%) and lowland (3%) (CSA, 2007).

Dairy farming using improved breeds is a common practice in urban and peri-urban areas. The farming system is intensive that run small to large sized with up to 400

milking cows, most of which are Holstein-Friesian cross-bred cows. Jersey and local Borana breeds are also present in some farms. The animals are fed with the concentrate and grass and given tape water according to the ration formula of the respective farms. In most of the farms there is exercise time once in a day for the animals when the weather is conducive. In all the farms hand milking is practiced. Some of the farms have their own milk processing machineries while others sending to collection and processing centers.

In the municipal abattoir an average of 15 -20 cattle are slaughtered every day except Tuesday and Thursday. Unscheduled slaughter, which include small ruminants, cattle and calves are conducted up on the demand from their customers. The abattoir has main slaughter hall with one over head rail. The rail is higher and serves for both small ruminant and cattle slaughter operation. In addition the abattoir has one emergency slaughter hall, one detaining room, and four chilling rooms. Furthermore, head, skin and gastrointestinal content collection rooms are available separately. The slaughtering process involved stunning and hoisting on to the over hail, bleeding and front leg removal hind leg removal, followed by de-hiding, splitting of sternum, evisceration and carcass splitting. Carcasses were pushed to the next process by the last person to complete the last operation. Hand washing was performed using tape water after pushing the carcass to the next operation. Carcass washing was conducted only when accidental spillage of cut contents occurred during evisceration. Finally the carcass is distributed to the owners by using special car of the municipal.

Asella

Asella: is the capital of Arsi zone, and is located in Oromia National Regional State, South Eastern Ethiopia, at about 175 km Southeast of Addis Ababa and has latitude and longitude of 7°57′N 39°7′E with altitude ranges from 2500 to 3000 metre above sea level. It has a human population of about 67,269. Monthly temperature variation is very little, due to its location close to the equator and the seasons are only distinguished by the intensity of rain, which is the most in August and the least in December (http://en.wikipedia.org/wiki/Asella).

Agricultural production system of the study area is of mixed crop and livestock production. Dairy farming using improved breeds is a common practice in urban and peri-urban areas (KARC, 2008). The farming system is semi-intensive that run small to medium sized with up to 100 milking cows, most of which are cross- breed among Holstein-Friesian, Jersey and local Arsi breed introduced by the artificial insemination program and exotic breeds, since the establishment of CADU (Chilalo Agricultural Development Unit) in the mid- 1960s by the Swedish- funded integrated rural development in Africa (Halderman, 2004). The animals in the intensive farms are managed in the same way as mentioned in Bishoftu above. In some of the farms animals free graze and confined at night. In all the farms hand milking is practiced. Milk processing is not common except in one government farm; it is sold raw to the customers. The number of the number of animals slaughtered, management, the slaughter process and the facility in the municipal abattoir of Asalla is almost similar with that of Bishoftu, except that 'Halla' method of slaughter is common here.

Addis Ababa

Addis Ababa is the capital city and administration centre for the Federal Democratic Republic of Ethiopia with total area of 527 km². Currently there are 10 sub-cities ("*KifleKetemas*") in Addis Ababa city administration delineated on the basis of geographical set up, population density, asset and service providers' distribution and convenience for administration (AACA, 2004). Addis Ababa is situated at latitude of 9°3'North and 38°43'East (ILCA, 1994). It lies in the central highlands of Ethiopia at an altitude of 2500 meters above sea level. It has an average rainfall of 1800 mm per annum. The annual average maximum and minimum temperature is 26°C and 11°C, respectively; with an overall average of 18.7°C. Highest temperatures are reached in May. The main rainy season extends from June to September. Addis Ababa has a relative humidity varying from 70% to 80% during the rainy season and 40% to 50% during the dry season. The human population is estimated at about 3.385 million (CSA, 2007).

Dairy farming is common in the urban and per- urban area of the city. Most of the farms nearly in the city are intensive that run small to medium sized with up to 300 milking cows, most of which are cross- breed among Holstein-Friesian, Jersey and different local breeds and these in the periphery are semi-intensive containing up to 100 milking cows. The management of the animals in the farms is similar to other areas. Machine processing of milk is common here but animals are hand milked.

The Addis Ababa abattoirs enterprise the largest slaughter house in the country is the only abattoir in the city. Hundred up to thousands of cattle are slaughtered here every day depending on seasons. The abattoir has every infrastructure for slaughtering of animals (cattle, sheep, goat, camel, swine, and calf). The way slaughtered animals are managed and the slaughtering process is similar to other abattoirs mentioned above, except machine splitting of carcass is common here.

Adama

Adama: is one of the largest and most populated towns in Oromia National Regional State. The current total population of Adama is 155, 321. It is in located in the Rift Valley, about 95 Km southeast of Addis Ababa (39.17°E and 8.33°N) with an altitude of 1622m above sea level. It receives an annual rainfall ranging from 400 to 800mm. The temperature range is 13.9 to 27.7°C (NMSA, 2006). The small-scale urban farmers in Adama breed several livestock types in traditional style and also intensive large scale dairies with improved breeds being operated with improved breeds. The town has also municipal abattoir where 20- 200 cattle are slaughtered per a day. The management of animals in the farms as well as slaughtering process in the abattoir is similar to above mentioned areas of selected towns of central Ethiopia.

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Holeta

Holeta: is located at 40km west of Addis Ababa and at an elevation of 2400 meters above sea level in the central Ethiopia. It has a total population of 25,593. The area is

characterized by mild subtropical weather, with average minimum and maximum annual temperatures of 6.3^{o}C and 22.1 ^{o}C, respectively. The area also experience bimodal rain fall pattern with a long rainy season extending from July to September while the short rainy season extends from March to April (CSA 2007). Semi-intensive animal farming is a common practice in the area, intensive farming with improved breeds as well. The town has also municipal abattoir where animals are slaughtered. The management of animals in the farms as well as slaughtering process in the abattoir is similar to above mentioned areas of selected towns of central Ethiopia.

3.1.2. Study population

The study population consisted of apparently healthy slaughtered cattle at municipal abattoirs and lactating cows of dairy farms in central Ethiopia from December 2013 to June 2014. The cattle slaughtered in the slaughter houses in Bishoftu, Asella and Adama mainly originated from markets around Awash Park in the Rift Valley area, Arisi and Bale of Oromia and Afar region. Those cattle slaughtered in Addis Ababa abattoir were from different regions (South, Oromia, Afar and Amhara) while cattle slaughtered in Holeta municipal abattoir were from its surroundings. Animals are transported to the respective abattoirs on double decked trucks made for animal transportation or open trucks made for transportation of goods or driven on feet. After arriving at the slaughter houses, the animals stayed for 24 to 72 hours in concrete floored roofed shades where they were fed and watered till they were slaughtered. Animals to be slaughtered the next day were inspected by veterinary inspectors and moved in to another lairage where they spend the night, feed being withheld since separation from other animals. The lactating cows in the farms are mainly Holstein-Friesian cross-bred cows, Jersey, pure local breeds (Borana, Arsi, others) and local breeds cross bred with exotics. In intensive farms animals are confined in the barns, fed according to the ration formulation record of the respective farms and hand milked. Prior to some hours before milking, animals are given feed and water. Immediately before milking the udder is washed with water and dried. In most of the farms one towel is used to dry udder of many animals. Finally the milk is supplied to the customers either processed or raw.

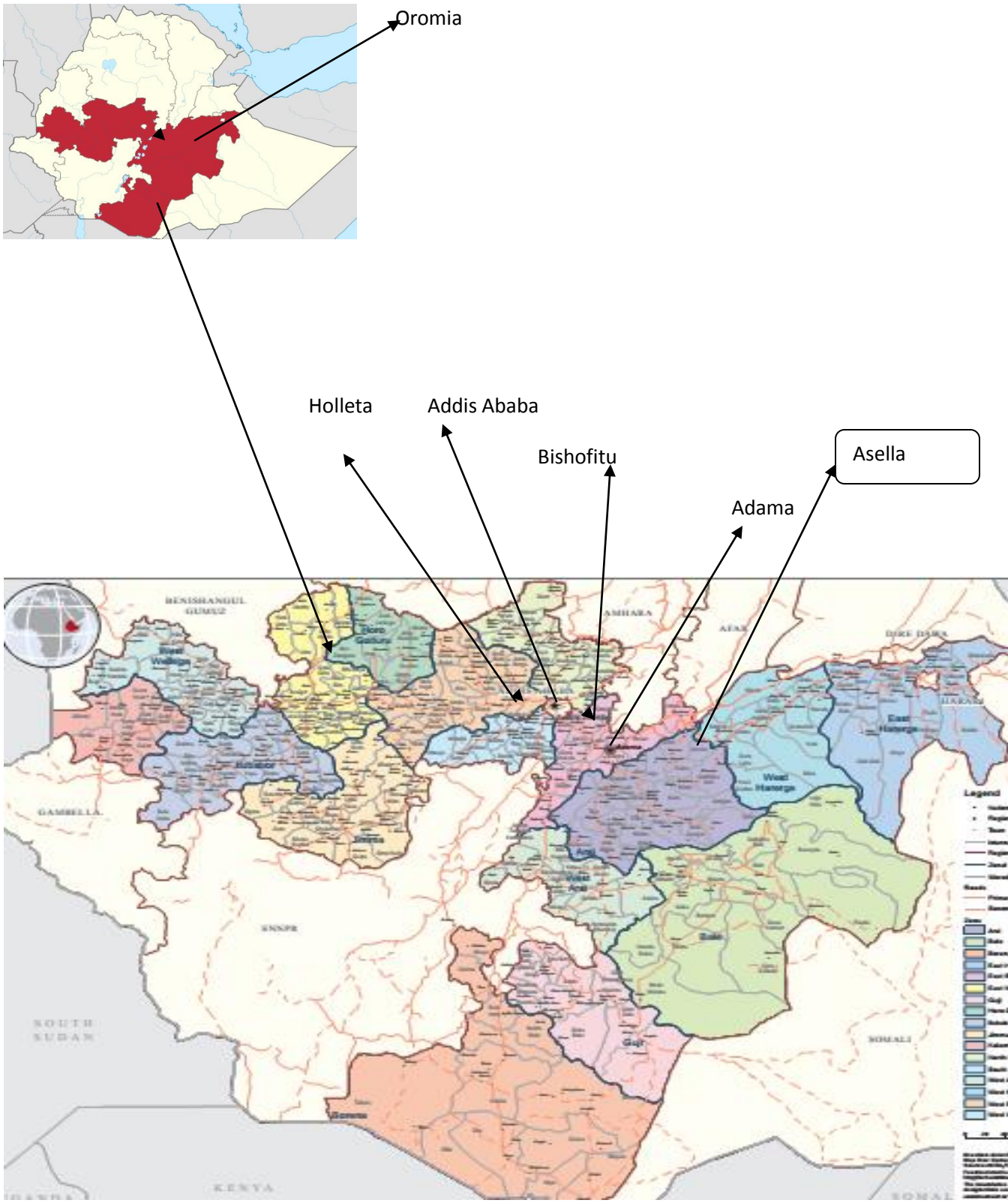

Figure 2: Map of central Ethiopia

3.2. Study design

A cross sectional study was undertaken in municipal abattoirs and dairy farms in central Ethiopia (Bishoftu, Asella, Adama, Addis Ababa and Holeta) from December 2013 to June, 2014. On each sampling day, usually once a week from each site, apparently healthy animals were randomly selected based on the number of slaughtered cattle in the abattoir and lactating cows on each farm, and sampled. Carcass *Salmonella* status was considered as an output variable of interest at slaughter houses and milk *Salmonella* status was considered as an output variable of interest in dairy farms. The explanatory variables considered in slaughter houses were *Salmonella* status of mesenteric lymph nodes, butchers' knives, cecal contents, carcass hanging materials and butchers' hands. The explanatory variables considered in dairy farms were milking buckets, milk collecting tanks, milkers' hands, tank milk and feces from lactating cows.

3.3. Sampling

Sample size required for this study was determined based on sample size determination in random sampling for infinite population using expected prevalence of salmonellosis and the desired absolute precision according to Thrusfield (2005) as follows

$$n = \frac{1.96^2 \, Pexp(1-Pexp)}{d^2}$$

Where

n=required sample size

Pexp=expected prevalence

d= desired absolute precision

A previous study on *Salmonella* in abattoirs at some towns of central Ethiopia and agro ecologically similar areas revealed an average prevalence of 9% (Alemayehu *et al*., 2003; Sibhat, 2006; Alemu, 2007). Therefore using 9% expected prevalence and 5% absolute

precision at 95% confidence level, the number of animals needed to estimate the prevalence of *Salmonella* in municipal abattoirs of central Ethiopia was calculated 126. A previous study on *Salmonella* in dairy farms at some towns of central Ethiopia and agro ecologically similar areas revealed an average prevalence of 15% (Zelalem *et al*., 2011; Teshome and Anbessa, 2012). Therefore using 15% expected prevalence and 5% absolute precision at 95% confidence level, the number of animals needed to estimate the prevalence of *Salmonella* in dairy farms of central Ethiopia was calculated 196. In actuality we have sampled more than calculated depending on the resource we had, however, sample types from farms and abattoirs were not uniformly taken because of constraints.

3.3.1. Sampling procedure

Sample animals from abattoirs were selected randomly and systematically using the identification number given for the animals both for the ante mortem and postmortem examination, depending on the number of animals slaughtered on each day, a number was drawn to fix the beginning of the sample and following slaughter order. Cecal contents, mesenteric lymph nodes, pooled carcass swabs from each selected slaughtered cattle, pooled carcass hanging materials swabs, pooled butchers' hand swabs and pooled butchers' knives swab samples were collected in separate sterile containers.

The pooled butchers' hand swabs were collected immediately before animals identified for sampling were eviscerated. Pooled butchers' hand swab means pooling hand swabs from different butchers eviscerating selected animals in to one. During each visit to abattoir we had one pooled butchers' hand swab. Both hands were swabbed from each butcher using a swab moistened with 10 milliliters (ml) of buffered peptone water (BPW). Lymph nodes with attached surrounding mesentery structures were removed by using sterile scissor and put in to sterile big universal bottles. After disinfection with 70% ethyl alcohol, the cecal wall was incised using a sterile disposable scalpel and approximately 25 grams (gms) of the contents were also collected aseptically. Knives used for evisceration were sampled before each selected animal was eviscerated and

finally pooled in to one sample, one pooled butchers' knives swab during each visit to abattoirs. Pooled carcass hanging materials swab was taken before carcass was hoisted on them.

Sterile cotton swabs moistened in 10ml buffered peptone water (BPW) were rubbed on the both sides of the carcass from hindquarter to forequarters uniformly. Swabbing of carcass was conducted holding the sterile sticks on opposite end of its tips. The swab samples were inserted in to the universal bottles containing buffered peptone water after cutting off the part of the stick which was in contact with the hand, by bending out on the mouth of the bottle. Carcass swab samples were collected at the end of slaughtering process before it is prepared for loading. At end of each sample collection, every sampling bottle was labeled including date of sampling and the type of sample collected corresponding to animal identification number.

Of the total farms, some (34) were selected randomly from a list of farms registered as milk producers in their respective municipal houses. Individual animal was selected randomly and systematically by using the number on its ear tag. Pooled swabs of the tanks were taken before milk is collected in to them, one pooled tank swab from each farm visited. Pooled swabs of buckets were taken immediately before they are used for milking. Pooled swab from milkers' hands were taken immediately before they start milking. Both hands from each milker were swabbed using a swab moistened with 10ml buffered peptone water. Approximately 25ml of milk was collected by milkers in a sterile universal bottle directly from the udder after the cows were restrained. Approximately 25gms of fecal specimens of cows were collected in a clean sterile air tight stool cup directly from the rectum. Samples collected from Addis Ababa, Holeta and Bishoftu were transported using an ice box to Addis Ababa University College of Veterinary Medicine and Agriculture microbiology laboratory. Samples collected from Adama and Asella were transported and analyzed at Asella regional veterinary diagnostic laboratory.

3.3.2. Sample processing

In laboratory lymph nodes were aseptically freed from the surrounding tissue, 25gm was weighed, immersed briefly in boiling water approximately for 10 seconds according to previously described method (Vieira-pinto *et al*., 2005). Each lymph node was then cut in to smaller pieces on the sterile petridishes by using sterile scalpel blade. The minced lymph nodes were put in to sterile stomacher bags and 225 ml of BPW was added and homogenized for two minutes with stomacher (Seward stomacher 400, London, UK). 25 grams of cecal contents, 25grams of feces and 25 ml of milk samples were put in sterile flasks individually. About 225ml BPW was added and the resulting mixture was agitated to disperse the contents. The mixing was done inside the safety cabinet. Test tubes containing swab samples in original 10ml BPW were shaken on a vortex mixer for 30 seconds for uniform distribution of microorganisms.

3.4. Isolation and identification

International organization for standardization (ISO) specifies food and animal feeding horizontal method for detection of *Salmonella* species should be based on the standard detection of *Salmonella* through different successive stages as shown in figure 1(ISO 6579; 2002). Therefore the isolation and identification was made based on this standard. Quinn *et al*. (1999) had been used to complement media preparation and in identifying colonies. The bacteriological media used in different stages were prepared according to the manufacture's recommendation (Appendix I).

3.4.1. Pre-enrichment in non- selective liquid media

Processed samples in appropriate amount of BPW (1:9) were incubated for 18h ± 2h at $37^{o}C \pm 1^{o}C$. Mesenteric lymph nodes were incubated while they were in stomacher bags whereas all the swabs, cecal contents and fecal samples were incubated in their original tubes and flasks (ISO 6579; 2002).

3.4.2. Enrichment in selective liquid media

Enrichment in selective liquid media was done by transferring 0.1 ml of culture obtained from the non-selective pre-enrichment media to a tube containing 10ml of Rappaport vassiliads *Salmonella* enrichment broth (Himedia laboratories Pvt.Ltd, Mumbai, India) and 1 ml to a tube containing 10 ml of Selenite F broth (Himedia laboratories Pvt.Ltd, Mumbai, India). The inoculated Rappaport vassiliads *Salmonella* enrichment broth was incubated at 42 ^{O}C for 24± 3h, Selenite F broth was incubated at 37^{O}C ± 1^{O}C for 24 ± 3h (ISO 6579; 1998: ISO 6579; 2002).

3.4.3. Plating out and identification

Xylose-lysine Deoxycholate agar (XLD) ((Himedia laboratories Pvt. Ltd, Mumbai, India) and SS (*Salmonella*, *Shigella* agar) (Oxoid LTD, Basingstoke Hampshire, England) plates were used for plating out and identification purpose. A loopfull of inoculums from Rappaport vassiliads *Salmonella* enrichment broth and Selenite F broth cultures was streaked on to XLD and SS agar plates and the inoculated plates were incubated at 37^{O}C for 24 ± 3h. After proper incubation, plates were examined for the presence of typical *Salmonella* colonies. Typical colonies of *Salmonella* grown on XLD medium produce hydrogen sulphide (H_2S) and have black (H_2S) centre and a lightly transparent zone of reddish color due to the color change of the indicator while on SS medium they become color less colonies with black centre. *Salmonella* H_2S negative variants (e.g. *S.* Paratyphi A) grown on XLD agar are pink with a darker pink centre whereas lactose- positive *Salmonella* are yellow with or without blackening (ISO 6579; 2002).

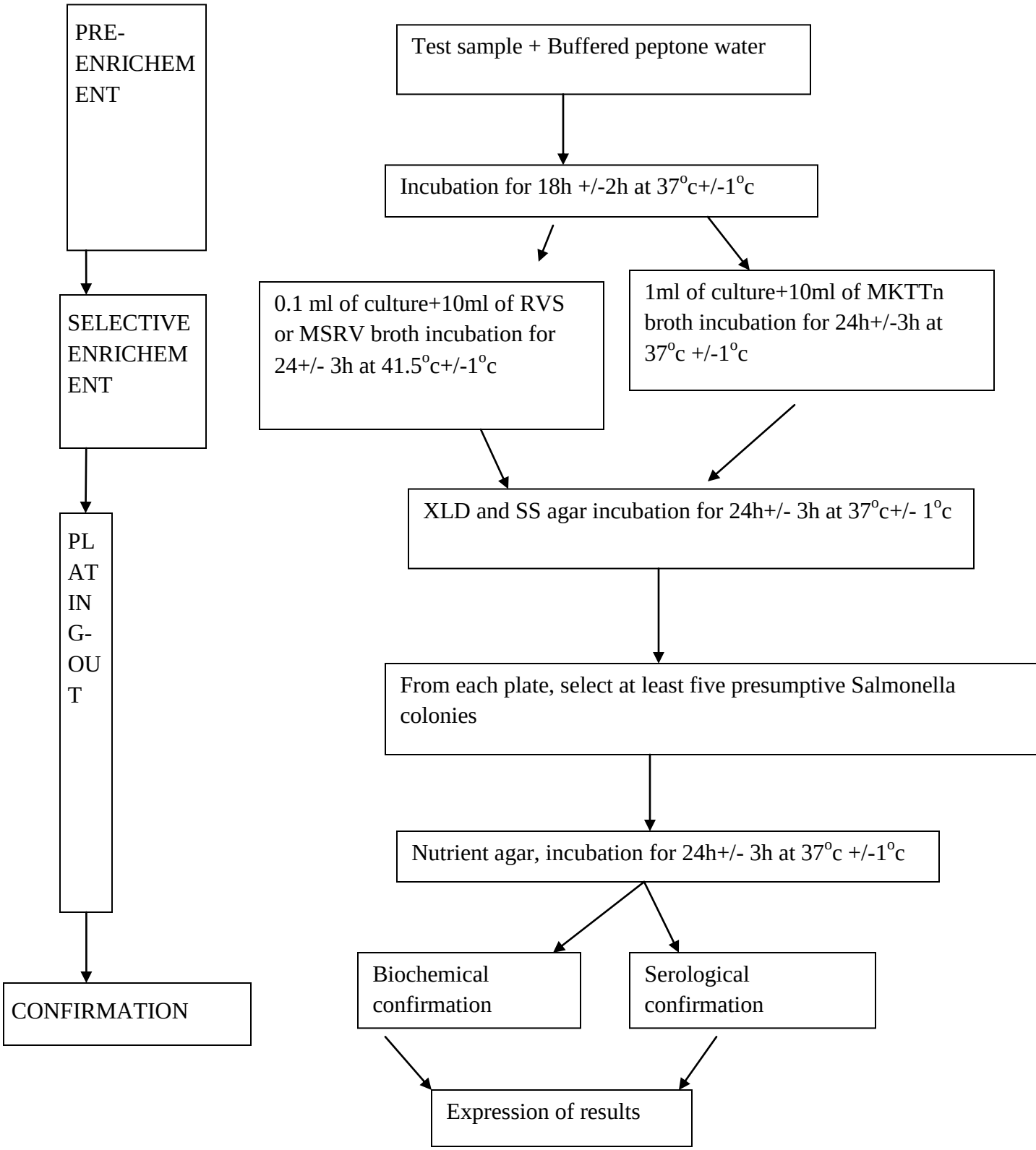

Figure 1: Horizontal method for detection of *Salmonella* (ISO 6579, 2002).

3.4.4. Confirmation

For confirmation, at least five presumptive colonies were selected from every selective plating media. Whenever the suspected colonies on each plate were fewer than five, all the colonies were selected. The selected colonies were streaked onto the surface of pre-dried nutrient agar (Himedia laboratories Pvt. Ltd, Mumbai, India) plates, in a manner that allow well isolated colonies to develop and incubated at $37^{O}C\pm 1^{O}C$ for 24 ± 3 h. Then the pure culture on nutrient agar was used for biochemical confirmation (ISO 6579; 2002).

Biochemical tests: pure culture obtained from nutrient agar were used for biochemical confirmation. Triple sugar iron agar (TSI) (Oxoid LTD, Basingstoke Hampshire, England) slants were inoculated from pure culture by streaking the slant and stubbing the butt and without flaming the wire lysine iron agar (LIA) (Difco^{TM}, Becton Dickinson) was inoculated just below the surface and both tubes were incubated at $37^{O}C \pm 1^{O}C$ for 24 ± 3h loosely capped to maintain aerobic condition and to prevent excessive production of H_2S production. Typical *Salmonella* culture in TSI agar show alkaline (red) slants, and acid (yellow) butts with gas (bubbles) formation and (in about 90% of the cases) formation of hydrogen sulphide (blackening of the agar). Alkaline reaction (purple color) both in the slant and the butt superimposed with H_2S after incubation indicates a typical positive reaction for *Salmonella* in lysine iron agar (ISO 6579; 2002).

Pure isolates were inoculated on urea agar (Himedia laboratories Pvt.Ltd, Mumbai, India) and Simmons's citrate agar (Himedia laboratories Pvt.Ltd, Mumbai, India) by streaking the slant. Both of the inoculated tubes were incubated at $37^{O}C \pm 1^{O}C$ for 24 ± 3h (ISO 6579, 2002) and change in the incubated media were interpreted for *Salmonella* after the end of incubation following the guide line. Positive reaction in urea changes the color of phenol red to rose pink and latter to deep cerise due to liberation of ammonia as the result of splitting of urea. However, typical *Salmonella* colonies do not hydrolysed urea; therefore the medium remain yellow (negative test) (ISO 6579; 2002). *Salmonella*

colonies on Simmons's citrate agar produce alkaline products using the medium as the sole carbon source hence deep blue color indicates positive reaction.

The pure isolates were also inoculated in to tryptose soya broth (TSI) (Oxoid LTD, Basingstoke, Hampshire, England) to determine the ability of an organism to spilt amino acid tryptophan to form the compound indole. For this an inoculated broth culture was incubated for $37^{O}C \pm 1^{O}C$ for 24 ± 3h and Kovac's reagent was added. In positive reaction pink colored ring is formed on the top of the broth due to reaction of indole with Kovac's reagent. However typical *Salmonella* colonies due not hydrolyze amino acid tryptophan; therefore the medium remain unchanged after addition of appropriate reagent (ISO 6579; 2002).

Finally pure cultures were inoculated in to MR- VP Medium (Buffered glucose broth) (glucose phosphate broth) (Himedia laboratories Pvt.Ltd, Mumbai, India), incubated at $37^{O}C \pm 1^{O}C$ for 24 ± 3h and broth culture in each incubated tube is divided in to two halves. One half is used for methyl-red test and the other is for Vogus-Proskauer test. Alpha-naphtol (5%) and potassium hydroxide (40%) were added to the medium prepared for vages-proskauer test to know where the organism utilize glucose though butylene glycol pathway and produce acetoin. If acetoin is present the medium will turn pink-reddish in color. Methyl red reagent is added in another half. If the organism uses the mixed acid fermentation pathway and produces stable acidic end-products, the acids will overcome the buffers in the medium and produce an acidic environment in the medium. When methyl red is added, if acidic end products are present, the methyl red will stay red. Typical *Salmonella* isolates are VP negative and MR positive (ISO 6579; 2002).

3.4.5. Antimicrobial resistance test

Isolates biochemically confirmed as *Salmonellae* were tested for their resistance to individual antimicrobial drugs by disc diffusion technique (CLSI, 2012). Four to five well isolated colonies grown on nutrient agar were transferred onto tubes containing 5 ml of typtic soy broth (Oxoid LTD, Basingstoke, Hampshire, England). The broth culture was

inoculated at 35^{O}C for 4hrs until it achieves or exceeds the 0.5 McFarland turbidity standard (appendix II). For these tubes which exceeded the turbidity standard, adjustment were made by adding the sterile saline solution to obtain turbidity visually comparable to the standard.

With in 15 minute after adjusting the turbidity of the inoculums suspension, a sterile swab was immersed in each of dilution suspension and swabbed uniformly over surface of two plates of muller Hinton agar (Oxoid LTD, Basingstoke, Hampshire, England) for each inoculums. The plates were held at room temperature for 30 minutes to allow drying. Using sterile forceps, discs impregnated with known concentration of antimicrobials were dispensed onto the surface of Muller Hinton agar plates. The plates were incubated at 37^{O}C for 20 hrs and examined for zone of inhibition. The diameter of zone of inhibition were recorded to the nearest millimeter, and classified as resistance, intermediate, or susceptible according to published interpretive chart (CLSI, 2012). The type of tested antimicrobials, their concentration in the discs and the zone of inhibition in deciding susceptibility are given in table 1

Table1: Antimicrobials and concentrations used to test susceptibility of *Salmonella* isolates (CLSI, 2012).

Antimicrobial agents and symbols	Disc contents(µg)	Zone diameter, nearest whole mm		
		Resistance	Intermediate	Susceptible
Ampicilline (AMP)	10	≤ 13	14-16	≥ 17
Erytheromycin (E)	5	≤ 13	14-22	≥ 18
Gentamycin (CN)	10	≤ 12	13-14	≥ 15
Kanamycin (K)	30	≤ 13	14-17	≥ 18
Streptomycin (S)	10	≤ 11	12-14	≥ 15
Trimethoprim-Sulfamethoxazole(SXT)	1.25/23.75	≤ 10	11-15	≥16
Amoxacillin (AML)	20	≤ 13	14-16	≥ 17
Tetracyclin (TE)	30	≤ 11	12-14	≥ 15
Chloramphenicol (C)	30	≤12	13-17	≥ 18
Cefoxitine (FOX)	30	≤ 14	15-17	≥ 18
Ciprofloxacin (CIP)	5	≤ 14	15-17	≥ 21
Nalixic acid (NA)	30	≤13	14-18	>19

3.5. Data management and analysis

All data were entered in to a Microsoft Excel spread sheet. After validation, the data were imported to STATA version 11 for windows (stata corp. College station, TX, USA) for descriptive analysis and association tests.

The prevalence of *Salmonella* at sample and animal level was expressed as percentage, with 95% confidence interval (CI), of total number of samples or animals positive to *Salmonella* to the total number of samples or total number of animals examined. An animal in abattoir was considered positive when mesenteric lymph node or cecal content or both samples were culture positive for *Salmonella*. In a farm an animal was considered positive when it was culture positive for *Salmonella* from feces.

The data was analyzed by comparing proportions using Pearson's chi-sqaure. For the association of risk factors in the abattoir with carcass contamination and in the farm with milk contamination, univariate logistic regression analysis was made. The explanatory variable in the abattoir (mesenteric lymph node, cecal content, pooled butchers' knives swab, pooled carcass hanging materials swab and pooled butchers' hand swab *Salmonella* status) and explanatory variable in farm (feces, tank swab, tank milk, pooled buckets swab, pooled milkers' hand swab *Salmonella* status) were separately analyzed to see their association with the outcome of the bacteriological status of the carcass.

4. RESULTS

The present study was conducted on 133 apparently healthy slaughtered cattle and 202 milking dairy cattle at central Ethiopia (Addis Ababa, Bishoftu, Adama, Holeta and Asella) from December 2013 to June 2014 to estimate the prevalence and distribution; assess antimicrobial resistance pattern of *Salmonella* and find out some of the risk factors of carcass and milk contaminations with *Salmonella*. Bacteriological examination was conducted on 984 different samples from dairy farms (udder milk, tank milk, bucket swabs, tank swabs, milker's hand swabs, fecal samples from lactating animals) and abattoir (carcass swabs, knives swab, butcher's hand swabs, cecal contents, carcass hanging materials swab and lymph nodes).

4.1. Prevalence of *Salmonella*

Out of the total 335 animals (133 slaughtered cattle, 202 lactating cows) examined for bacteriological status of *Salmonella*, 29 (8.7%) were positive of these, 13(9.8%) were slaughtered cattle and 16 (7.92%) were lactating cows. No statistically significance difference ($p>0.05$) were found between slaughtered cattle and lactating cows in being positive for *Salmonella.* Slaughtered cattle was considered *Salmonella* positive when it was bacteriologically positive either from mesenteric lymph node and/cecal content. Likewise, lactating cow was considered *Salmonella* positive when it was bacteriologically positive from feces. Carcass status of slaughtered cattle and milk status of lactating cows were considered indicators of contamination and were not used for calculation of prevalence. None of the slaughtered cattle were positive both from the mesenteric lymph node and cecal content.

4.2. Isolation of *Salmonella* from different sources

A total of 984 different samples from farms and abattoirs of central Ethiopia were bacteriologically examined for presence of *Salmonella. Salmonella* was isolated from 66(6.71%; 95%CI: 5.14%, 8.27%). Out of 984 samples 439 were originated from

abattoirs and 545 were from the farms. The prevalence from abattoirs and farms were 9.11% (40/439) (95% CI: 6.41%, 11.81%) and 4.77% (26/545) (95% CI: 2.98%, 6.56%) respectively. There was significance ($P<0.05$) difference in the isolation frequency of *Salmonella* from abattoir and farm. *Salmonella* was two times more likely isolated from abattoirs than farms (OR= 2.00 (95%CI: 1.20 – 3.33, p=0.008).

4.2.1. Isolation of Salmonella from farm Samples

Of the total of the total of 545 samples originated from the farms, *Salmonella* was isolated from 26 (4.77%) samples of which 16(7.92%) feces, 1(2.94%) pooled buckets swab, 1(2.94%) pooled milkers' hand swab, 3(8.82) pooled tank swab, 1(2.94%) tank milk and 4(1.91) udder milk samples ware positive for *Salmonella* (Table, 3).

Table 2: Prevalence of *Salmonella* in different sample types from farms in central Ethiopia

Sample type	Number examined	Positive (%)	95%CI	X^2	P- value
Feces	200	16(7.92)	4-11.8	10.3264	0.067
Pooled buckets swab	34	1(2.94)	1-8.7		
Pooled milkers' hand swab	34	1(2.94)	1-8.7111		
Pooled tank swab	34	3(8.82)	1-18.5		
Tank milk	34	1(2.94)	1-8.7		
Udder milk	209	4(1.91)	1-3.8		
Total	545	26(4.77)	2.96-6.56		

Salmonella was detected in all test samples obtained from farms with different frequencies of occurrence. There were no statistically significant differences (p> 0.05) in the proportion of *Salmonella* among farm sample types.

The level of milk contamination was considered as an outcome variable taking feces, pooled buckets swab, pooled milkers' hand swab and pooled tank swab as a risk factors for milk contamination. Therefore, association of milk contamination with the risk factors was assessed using univariate logistic regression analysis (Table, 4) and no statistically significant associations was demonstrated between the milk contamination and milkers' hand swab, pooled buckets swab and tank milk (p>0.05). However, feces and tank swab were found to be significantly associated with milk contamination and the ORs were 4.45 and 5 respectively. Therefore a carrier animal is 4.45 times more likely to contaminate milk with *Salmonella* compared with a *Salmonella* free animal. In addition milk stored in *Salmonella* positive tanks is five times more likely to be contaminated with *Salmonella* compared with milk stored in *Salmonella* negative tanks.

Table 3: Results of univariate logistic regression analysis for association of milk contamination with the risk factors.

Risk factors	Odds ratio	95%CI for the odds ratio	P-value	Std.Err
FC	4.45	1.46 - 13.57	0.009	2.53
BS	1.55	1-14.33	0.7	0.39
TS	5	1-23.22	0.042	3.9
TM	1.55303	1-14.33	0.7	0.39
MHS	1.55303	1-14.33	0.7	0.33

FC =feces, BS= pooled buckets swab, TS= pooled tank swab, TM=tank milk, MHS= pooled milkers' hand swab

The highest prevalence (7.6%) was detected at Bishoftu while the lowest (3.2%) was in Holeta. However, no statistically significant differences (P>0.05) was observed among the study areas.

Table 4: over all prevalence of *Salmonella* in farms in selected towns of central Ethiopia

Area	Sample examined	Positive (%)	X^2	P-value
Addis Ababa	92	4(4.4)	3.84	0.43
Bishoftu	144	11(7.6)		
Asella	91	4(4.4)		
Adama	92	3(3.3)		
Holeta	126	4(3.2)		
Total	545	26(4.77%)		

As to the overall proportion of *Salmonella* in farm samples of different agro ecology, 4.5% was isolated from highland (Addis Ababa, Holeta and Asella) and 5.1% from mid land (Bishoftu and Adama). No statistically significant differences ($p>0.05$) was found between two agro ecologies to the prevalence of *Salmonella* ($p=0.266$; $X2=1.2362$).

4.2.2. Isolation of Salmonella from abattoir Samples

Of the total of 439 samples originated from the abattoirs, *Salmonella* was isolated from 40 (9.11%) samples of which the highest prevalence was found in pooled carcass hanging materials swab (23.80%).

Table 5: Prevalence of *Salmonella* by sample types from abattoirs

Sample type	Number examined	Positive (%)	95%CI	X^2	P- value
CS	183	13(7.10)	3.4-10.8	13.50	0.061
CC	133	7(5.34)	1.4-9.1		
BS	26	4(15.38)	1.2- 29.5		
KS	28	5(17.85)	3.3-32.3		
HS	21	5(23.80)	5.1-42.5		

LN	47	6(12.76)	3.1-22.4
Total	439	40(9.11%)	6.41 - 11.81

CS= carcass swab, CC= cecal content, BS= pooled butchers' hand swab, KS=pooled butchers' knives swab, HS= carcass hanging materials swab, LN= mesenteric lymph node.

Salmonellae were isolated from all test samples obtained from abattoir with different frequencies of occurrence. There were no statistically significant differences ($p>0.05$) in the proportions of *Salmonella* between samples of abattoir origin.

The level of carcass contamination was considered as an outcome variable taking cecal content, pooled knives swab, pooled carcass hanging materials swab, pooled butchers hand swab and mesenteric lymph nodes as a risk factors for carcass contamination. Therefore, association of carcass contamination with the risk factors was assessed using univariate logistic regression analysis (Table,7) and statistically significant association was demonstrated between carcass hanging materials and carcass contamination ($p=0.017$), but no significant association was demonstrated between other risk factors and carcass contamination ($p>0.05$). Therefore carcass hoisted on *Salmonella* positive hanging material is four times more likely to be contaminated with *Salmonella* compared to carcass negative hanging materials.

Table 6: Results of univariate logistic regression analysis for association of carcass contamination with risk factors

Risk factors	Odds ratio	95%CI for the odds ratio	P-value	Std.Err
BS	2.27	1-7.6	0.18	1.34
HS	4.09	1.3 – 13	0.017	2.40
LN	1.91	1-5.33	0.215	1.24
KS	2.84	1-8.71	0.067	1.83
CC	1	0-1.87	0.5	0

CS= carcass swab, CC= cecal content, BS= pooled butchers' hand swab, KS=pooled knives swab, HS= carcass hanging materials swab, LN= mesenteric lymph node.

Salmonellae were isolated from abattoirs in all the selected towns of central Ethiopia with proportion of 10.2% from Addis Ababa, 8.5% from Asella, 8.3% from Bishoftu, 8.9% from Adama and 9.0% from Holeta. Chi-square analysis revealed that there was no statistically significant differences (P>0.05) between selected towns of central for being positive for *Salmonella* in abattoir samples.

Table 7: Prevalence of *Salmonella* in abattoirs in selected towns of central Ethiopia

Area	**Sample examined**	**Positive (%)**	**X^2**	**P-value**
Addis Ababa	108	11(10.2)	0.2187	0.99
Asella	94	8(8.5)		
Bishoftu	47	4(8.3)		
Adama	90	8(8.9)		
Holeta	100	9(9.0)		

As to the proportion of *Salmonella* in the abattoirs on basis of agro ecology; the overall prevalence of 9.3% was isolated from highland agro-ecologys (Addis ababa, Asella and Holeta) and 8.8% from mid lands (Adama and Bishoftu). No statistical significance (p>0.05) was observed in proportion of salmonella based on agro ecology (p=0.863; X^2).

4.3. Antibiotics susceptibility profile of *Salmonella* isolates

All of the isolates obtained from the study (n=66) were tested for ten different antimicrobials that were commonly used in human as well as animal treatment, and available in the market. Fifty six of the total 66 isolates (84.8%) were resistant to one or more of the tested antimicrobials. Of the resistant isolates, 87.5% (49 of 56) of them developed resistant to more than one drug.

All the isolates were susceptible to the antimicrobial effect of Ciprofloxacin, 97% to gentamycin and 75.8 to sulphamethozonetrimethoprim and 60.6 % to nalidixic acid. The isolates showed high resistance to amoxicillin 60.6%), cefoxitin (57.6%), ampicillin (56.1%), streptomycin (48.4%), chloramphenicol and kanamycin (37.9%).

Table 8: Antibiotic susceptibility result of *Salmonella* isolates

Drug type	No of isolates	No of resistant isolates (% &95%CI)	No of intermediate isolates (%)	No of susceptible isolates (%)
Kanamycin (30µg)	66	25(37.9; 95%CI: 25.9-49.9)	8(12.1;95%CI:4-20)	33(50; 95%CI:37.6-62.4)
Nalidixic acid (30µg)	66	18(27.3; 95%CI:16.2-38.3)	8(12.2; 95%CI:4-20)	40(60.6; 95%CI: 48.5-72.7)
Gentamycin (10 µg)	66	1(1.5;95%CI:0-4)	1(1.5; 95%CI: 0-4)	64(97;95%CI:92.7-100)
Cefoxitin (30 µg)	66	38(57.6; 95%CI: 45.3-69.8)	-	28(42.4; 95%CI:30.1-54.6)
Streptomycin(10 µg)	66	32(48.4; 95%CI: 36.1-60.9)	6(13.6; 95%CI: 5.1-22.1)	25(37.9; 95%CI: 25.9- 49.9)
Chloramphenicol (30 µg)	66	25(37.9; 95%CI: 25.9-49.9)	4(6; 95%CI:0-12)	37(56.1; 95%CI: 43.8-68.3)
Sulphamethohazole trimethoprim (25 µg)	66	3(19.7; 95%CI: 9.8-29.5)	3(4.5; 95%CI: 0-9.7)	50(75.8; 95%CI: 65.1-86.4)
Amoxicillin (25 µg)	66	40(60.6; 95%CI:48.5-72.7)	-	26(39.4; 95%CI: 27.3-51.5)
Ampicillin (10 µg)	66	37(56.1; 97%CI: 43.8-68.4)	9(13.6; 95%CI: 5.1-22.1)	20(30.3; 95%CI: 18.9-41.7)
Ciprofloxacin(5 µg	66			66(100;

Total of 39 multidrug resistance (MDR) patterns were observed. The highest MDR noted was FOX, AML, AMP (7.6%, 5/66). The maximum MDR registered was resistance to eight antibiotics with the combination K,NA,FOX,S,C,SXT,AML,AMP being more frequent (Table 10). In general, MDR to two (13.7%, 9/66) and three (24.3%, 16/66) antibiotics dominate the resistance patterns. No difference in antimicrobial susceptibility patterns were observed in any *Salmonella* isolates from different sample type and different areas.

Table 9: Multi drug resistant pattern of *Salmonella* isolates from dairy farms and abattoirs located in central Ethiopia

Number of antimicrobials	Antimicrobial resistance pattern (number of isolates)	Number of isolates (%)
Two	NA,S (1)	9(13.6)
	K,NA(1)	
	FOX,AML(4)	
	AML,AMP(1)	
	FOX,S(1)	
	K,AMP(1)	
Three	N,S,AMP(1)	16(24.2)`
	K,S,AMP(1)	
	K,FOX, AML(1)	
	K,FOX,S (1)	
	S,C,AML(1)	
	S,AML,AMP(1)	
	FOX,S,AML(3)	
	FOX,AML,AMP (5)	
	FOX,S,AMP(1)	
	K,NA,AML(1)	
Four	N,FOX,S,AMP	8(12.1)
	K,NA,AML,AMP	

	FOX,S,AML,AMP	
	FOX,S,C,AML	
	FOX,SXT,AML,AMP	
	K,C,AML,AMP	
	K,NA,S,C	
	FOX,C,AML,AMP	
Five	K,FOX,S,AML,AMP	7(10.6)
	FOX,S,C,AML.AMP	
	K, NA,SXT,AML,AMP	
	K,NA,FOX,AML,AMP	
	K,C,SXT,AML,AMP	
	NA,S,C,AML,AMP	
	FOX,S,C,SXT,AMP	
Six	K,FOX,S,C,AML,AMP	2(3.0)
	K,NA,FOX,C,SXT,AMP	
Seven	K,NA,FOX,C,SXT,AML,AMP (4)	4(6.0)
Eight	K,NA,FOX,S,C,SXT,AML,AMP(3)	3(4.5)

AML=Amoxicillin; AMP=Ampicilline; CIP=Ciprofloxacin C=Chloramphenicol; CN=Gentamycin; FOX=Cefoxitine; K=Kanamycin; NA=Nalidixic acid; S=Streptomycin; SXT= Trimethoprim-Sulfamethoxazole

5. DISCUSSION

5.1. *Salmonella* prevalence

In the present study in central Ethiopia, 9.8% of apparently healthy lactating cows carried *Salmonella* in their fecal contents. The findings are in agreement with the report of Zelalem *et al*. (2011), which was 10.76%. This high proportion of carrier state could be associated with the fact that salmonellosis is more prevalent in areas with intensive animal husbandry, where animals live in a confined environment (Wray and Davis, 2003). *Salmonella* shed in the feces of carrier cows can contaminate milk during the milking process (Randall, 2001). In recent study, univariate logistic regression analysis for association of milk contamination with risk factors revealed that a carrier animal is 4.45 times more likely to contaminate milk compared to a *Salmonella* free animal. Even though most of the reports are on slaughtered cattle from abattoirs and ready to eat food items, lactating cows could be potential sources of *Salmonella* infection for individuals working in dairy farms and for the community at large (Zelalem *et al.*, 2011).

In the present study, the prevalence of *Salmonella* in milk directly taken from the udder was 1.9%. The finding is comfortably comparable with Zelalem *et al.* (2011) who reported 3.1% prevalence in milk from dairy farms of Addis Ababa. Evidences (Hitoshi, 2006; Mahami *et al*., 2011) also indicated that *Salmonella* spp are agents for the cause of mastitis in dairy animals and may have contaminated milk from the udder of infected animals and also reside in the intestinal tract where they cause gastro-enteritis in animals and may have occurred in milk as a result of faecal contamination.

In the tank milk, 2.9% prevalence of *Salmonella* was also detected in the present study. The finding is in consistent with 2.24% (Steele *et al.*, 1997) prevalence of milk samples from the bulk tanks in USA farms. However, it is higher than 0.17% prevalence in bulk tank samples from Ontario, Canada and lower than 4% from Iraq (Forough, 2012) and by far lower than 20% report from Kersa district of Jimma, Ethiopia (Teshome and

Anbessa, 2012). Large variation between our finding and reported prevalence from Jimma could be associated with sampling procedures, sampling plan or bacteriological techniques employed in detection of *Salmonella*. Generally from these studies it is clear that raw milk consumption and consumption of products made with raw milk can present some risk. Although proper pasteurization reduces this risk to public, a large group of people consume raw milk in Ethiopia. The levels of *Salmonella* in the milk samples (udder and tank milk) tested here seemed to be very low, however, the infectious dose for this organism is as low as 15 to 20 cells (Forough, 2012). The potential for this organism to grow in improperly stored raw milk and in products made from raw milk presents a public health risk, particularly to susceptible members of the population.

In our study, 2.9% and 8.82% prevalence of *Salmonella* were observed from pooled swabs of buckets and tanks respectively. Though, no comparable data is available for buckets used to milk and tanks used to store the milk but recent finding pin pointed the importance of storage and handling materials for milk contamination with *Salmonella*. The univariate logistic regression for association of milk contamination risk factors revealed that milk stored in *Salmonella* positive tanks is five times more likely to be contaminated with *Salmonella* compared with milk stored in *Salmonella* negative tanks. In addition Teshome and Anbessa, (2012) described the storage material and ways of handlings as important sources of *Salmonella* contamination of the raw milk. CDC (2004) also described that *Salmonella* likely contaminated the containers or milk contact surfaces because of environmental conditions in the plant. *Salmonella* are wide spread in the environment and can exist in many niches. Farm utensils may become directly contaminated with *Salmonella* following outbreak of disease or colonization of animals, or indirectly from other sources such as contaminated water used for cleaning or farm attendants. Moreover, milk residue left on equipment contact surfaces supports the growth of a variety of microorganisms (Murphy and Boor, 2010). Therefore milking equipment should be designed, constructed, installed, maintained and used in a manner that will avoid the introduction of contaminants into milk. It also should be thoroughly cleaned with potable water immediately after use and before use.

In this study, another low proportion of *Salmonella* was isolated from pooled hand swabs of milkers, 2.94%. The finding is in agreement with 3.1% prevalence from stool in food handlers reported from Gondar (Legesse *et al.*, 2014) and 3.4% from Addis Ababa (Fentabil, 2011). However, it varies considerably from other previous reports. Zelalem *et al.* (2011) reported 13.63% prevalence in stool from individuals working in dairy farms of Addis Ababa. Zewudu and Cornelius, (2009) reported 7.6% in supermarket personnels in Addis Ababa. Furthermore, Alemayehu *et al*, (2003) reported 6% from slaughter house personnel. The disparity of findings could be due to the difference in the type of sample taken. Working environment and different in hygienic status can also be considered. The presence of *Salmonella,* which is infective in very low dose, on the hands of milkers immediately before milking is a concern to the dairy farms that provide milk and milk products to the community since cross contamination from these individuals could be a potential source of food borne infections.

The current study also revealed that 7.92% of slaughtered cattle in central Ethiopia carried *Salmonella* in their cecal contents or mesenteric lymph nodes. The result is in agreement with the report of Alemu (2007), who reported 7.1% of carrier state in Bahir Dar, Ethiopia. However, the prevalence in present finding is higher compared to a 3.8% in Addis Ababa abattoir (Alemayehu *et al.,* 2003) and lower compared to a 14% in Bishoftu abattoir (Sibhat, 2006). The difference in the prevalence could be associated with the bacteriological technique used in detecting *Salmonella* or the difference in hygiene of dressing operations or difference in the occurrence and distribution of *Salmonella* in the study population regardless of sample type and method of detection (McEvoy *et al.,* 2003). This significantly high proportion of *Salmonella* could be due to result of longer time that the cattle stay in the lairage before slaughter. It has been shown that a decrease in daily feed intake enhanced the growth of *Salmonellae* in the rumen and fecal excretion by carrier animals (Venter *et al*., 1994). The presence of *Salmonella* excretors in the batches of animals in transit and passing through the lairage could result in contamination of skins. The surfaces of carcasses are easily contaminated with *Salmonella* from skins in abattoirs with poor hygienic control during skinning and evisceration (Teklu, 2008).

In mesenteric lymph nodes, 12.76% detection rate was observed which is fairly comparable with 8% finding of Sibhat (2006) in Bishoftu and 3.2% in Bahir Dar (Alemu, 2007) but higher than 4.5% previous report by Alemayehu *et al*. (2003) from Bishoftu. However, the current study finding is much lower than 91.2% in Unite states (Sara *et al*., 2013) and 57.1% in Australia (Samuel, 1980). It is surprising to see high prevalence of *Salmonella* in lymph nodes of slaughtered cattle in advanced countries where animals are usually immunized against *Salmonella* and the hygiene is peak. Despite effective control of *Salmonella* on carcass surface, over presentation of *Salmonella* in lymph nodes is an important data gap in developed countries. Our finding should also not be overlooked; it is very high for the pathogen which is infective in very low dose. The finding of high proportion of infected cattle harboring *Salmonella* in their mesenteric lymph nodes in this study indicate the existence of substantial risk of cross contamination possibly during slaughtering, subsequent handling, storage and distribution of the carcass. Meat inspectors and abattoir personnel should take care during slaughtering and incision for inspection in order not to contaminate carcasses through knives and hands. In addition, in Ethiopia, minced beef is usually used for the preparation of a popular traditional Ethiopian dish known as locally *''kitfo''* and most of the time it is consumed raw or medium cooked, therefore they should take care not to incorporate lymph nodes during preparation.

The prevalence of *Salmonella* from carcasses in this study was 7.10%. This is in agreement with the report of previous works. Neiyeleti *et al*. (2000) reported respective 9.8 and 11.9% prevalence of *Salmonella* on abdominal muscle and diaphragmatic muscle from cattle slaughtered at Addis Ababa abattoir and Molla *et al*. (2003a) reported 5.6% prevalence on carcass samples collected from Addis ababa and Bishoftu abattoirs. The finding was also in consistent with 7.6% (Muluneh and Kibret, 2015) and 4.8% (Alemu, 2007) reports from Bahir Dar abattoir. It was however higher than 2% (Sibhat, 2006) and 3.1% (Alemayehu *et al*., 2003) report from Bishoftu abattoir. The method of sampling could be a source of variation for the findings. The high level of carcass contamination with *Salmonellae* is of special public health significance in a country like Ethiopia, where

raw and under cooked meat is the favorite meal in most areas. The presence of even small numbers of *Salmonella* in carcass meat and edible offals may lead to heavy contamination of minced meat and sausage. When meat is cut into pieces, more microorganisms are added to the surfaces of exposed tissue (Ejeta *et al*., 2004).

Contamination of equipments and materials can spread pathogenic bacteria to non contaminated carcasses (Nouichi and Hamdi, 2009). This was evidenced by finding 23.80% prevalence of *Salmonella* from pooled swab of carcass hanging materials. The logistic regression analysis done to see the association of carcass contamination with hanging materials also revealed carcass hoisted on *Salmonella* positive hanging material is four times more likely to be contaminated with *Salmonella* compared to carcass negative hanging materials. When people clean the abattoir environment they don't usually clean the hanging materials which are high to be cleaned thoroughly; and *Salmonella*, a ubiquitous organism which can survive several weeks to years in the external environment can comfortably reside there. Therefore specific attention should be given to the sanitation of each and every equipment and materials in the abattoir.

Salmonella was also isolated from abattoir equipment, knives swab, with a prevalence of 17.85%. This is consistent with the 26.7% prevalence of knife blades in Botswana abattoir (Teklu, 2008). And also comfortable with finding of Teklu (2008) who recorded prevalence 7.4 of *Salmonella* prevalence in knives used to slaughter shoats in Modjo abattoir, Ethiopia.

Salmonella was recorded at a prevalence of 9% (Sibhat, 2011) in palm swabs of personnels involved flying and evisiration of cattle in Bishoftu abattoir and 8.8% from hand swabs of sheep and goat eviscerators in Modjo export abattoir, Ethiopia (Teklu, 2008). Our result (15.38%) was relatively comparable with these findings. However higher prevalence of *Salmonellae* (30%) were isolated from the hands of workers in all stage along the slaughtering line in Queensland. Therefore these uniform findings in Ethiopia in personnels involved in slaughtering process of sheep and cattle in different areas and abattoirs, in different times indicates that the hygienic condition of personnel

are the same and not getting improved. We also observed this during visit to different abattoirs in selected towns of central Ethiopia. In general there is association between cattle carcass and personnel. This was evidenced by Molla *et al*. (2003a) who isolated *Salmonella* serovars; S. Typhimurium, S. Anatum and S. Dublin from apparently healthy abattoir personnel and cattle carcass simultaneously.

Finally, *Salmonella* were uniformly recovered from different samples from different agro ecology. From farms respective 4.5% and 5.1% prevalence were recorded from high land and mid land. From abattoir respective 9.3% and 8.8% prevalence were recorded from high land and mid land. The finding reflects the physiological properties that it grows in the temperature range of 2- 47^{o}C, widely distributed in different agroecologies, survival of *Salmonellae* for prolonged period of time in environment (D'Aoust, 2000; 2001). Further more data from 37 countries that participated in World Health Organization Global Foodborne Infections Network evidenced uniform distribution of *Salmonella* serotypes in humans and animals samples (Hendriksen *et al*., 2011). Therefore the epidemiology of *Salmonella* is complex, it is wide spread bacterium in the farm and food animal environment that can cause significant problems in animal and human health. Suitable approach to hygienic condition should be practiced in farms and abattoirs so as to reduce contamination of food of animal origin with *Salmonella*.

5.2. Antimicrobial resistance

Antimicrobial resistant *Salmonella* isolates from animal and human sources have been reported in Ethiopia (Alemayehu *et al.,* 2003; Molla *et al.,* 2006; Legesse *et al*., 2014). In this study isolates were also recovered from animal and human source and tested against the panel of ten antimicrobials. Out of all 66 isolates tested 56(84.8%) found to be resistant to one or more antimicrobials. No significance difference in the antimicrobial resistance of isolates from different samples was observed (P>0.05). Our result compares favorably with 83.3% resistance report for isolates from abattoir (Dabassa and Bacha, 2012) and 83.3% from milk (Zelalem *et al.,* 2011). It was, however, higher than 70% of resistance reported from milk (Teshome and Anbessa, 2012) and 62.5% cattle meat

(Molla, 2004). The result is by far higher than 41.4% (Sibhat, 2006) and 39.3 % (Alemu, 2007) over all resistance reported for isolates from slaughtered cattle; 46.2% (Legesse *et al*., 2014) from food handlers and 44.8% (Molla *et al*., 2006) resistance reported for isolates from slaughtered camels.

When animals from which they originate have been treated with the particular antimicrobial over a long period, transmitted bacteria will have acquired resistance to specific antimicrobials (Alemu, 2007). Therefore the difference in the level of antimicrobial resistance from the previous study might be described from the view of differences in frequency and type of antimicrobials used in an area where the animals originated, or might arise from differences in bacterial culture and antimicrobial susceptibility testing technique (Padungtod and Kaneene, 2006). The difference observed might be also due to differences in the number of antimicrobial drugs tested in the present study and as well as diversity in their usage. In addition, in Ethiopia, the indiscriminate usage of antimicrobials for human as well as animal treatment is increasing from time to time and this might have been contributing to drug resistance. My observation during my visit in central Ethiopia was also the same. There was extensive uncontrolled circulation and indiscriminate usage of drugs used for animal and human treatment.

The results of antimicrobial susceptibility test in the current research revealed that 87.5% of 56 resistant isolates were multidrug-resistant according to the recent drug-resistance definition (Magiorakos *et al.,* 2012). This is among one of the highest multidrug resistance findings in Ethiopia. Multiple antimicrobial resistant among the human antimicrobial resistant *Salmonella* isolates in Ethiopia ranged from 0% reported by Fentabil (2011) from food handlers of Addis Ababa university Cafeteria to 88.5% by Beyene (2008) from stool of children; with results from other studies fitting between these values. Other results are like 46.2% multidrug resistant isolates from food handlers of University of Gondar (Legesse *et al*., 2014) and 50% from pediatric patients from Harar (Ayalu, 2011).

Most of reports by different researchers on the antimicrobial resistance of *Salmonella* in slaughtered animals and animal products in Ethiopia, indicated that the proportion of multidrug resistant isolates higher than isolates resistant to a single antimicrobial, ranging from 52.2% (Molla, 2004) to 100% (Dabassa and Bacha, 2012) with report of multidrug isolates by other investigators fall between these values. In addition respective 75% (Teshome and Anbessa, 2012) and 83.3% (Zelalem *et al*., 2011) multidrug resistant of *Salmonella* isolates from milk has been detected.

In general prevalence of multidrug resistance in recent finding is leading almost all the other findings and it is in line with the idea described by Bada *et al*. (2006) that as there is increasing resistance to commonly used antibiotics in animals and humans, there is also concurrent increase in multiple resistant *Salmonella* isolates worldwide from time to time. It has already been imitated that emergence of multiply resistant *Salmonellae* can be the product of conjugative transfer of R-plasmid between bacterial species. Agricultural use of sub therapeutic doses of a single antibiotic could select for bacterial strains harboring plasmids with multiple resistant codons. The emergence and prevalence of multiply resistant *Salmonellae* in meat and milk animals can seriously compromise public health (Molla, 2004).

In current study the maximum MDR registered was resistance to eight antibiotics with the combination K, NA, FOX, S, C, SXT, AML and AMP. This is in line with multiple drug resistance up to nine antimicrobials containing AMP, AML, S, C, SXT, FOX, SPT, TMP and SUL (Molla, 2004). Likewise MDR up to six antimicrobials with the combination of AMP, S, TMP, SUL, SXT and SPT was recorded (Tibaijuka *et al*., 2002). The detection of multidrug resistant as many as combination of eight antimicrobials in this study, indicates public health significance of these isolates as contaminated cattle milk, milk products, meat and meat products may pose health hazards. The high risk parts of human population that is infants, elderly, immunocompromised such as individuals with HIV/AIDS and malnourished persons are highly susceptible and a presence of *Salmonella* even in a low number constitute a major public health concern (Molla, 2004). This risk can further be accentuated if milk and milk products are

consumed raw or unpasteurized and meat and meat products are consumed raw or under cooked.

Further analysis of antimicrobial susceptibility test results showed that, in present study the *Salmonella* isolates are most susceptible to Ciprofloxacin (100%).This result was similar with the result reported by (Teshome and Anbessa, 2012) from Jimma, Ethiopia, among isolates from raw cow milk. The finding is also in line with result reported by Zelalem *et al*., (2011) from Addis Ababa, among isolates from lactating cattle and contact personnel and with that reported by Molla *et al*. (2006) from central part of Ethiopia among isolates of sheep and goat. It was also comparable result reported from Nigeria by Akinyemia *et al*. (2005). The data from (Dabassa and Bacha, 2012) has indicated that, the effectiveness of such drugs like ciprofloxacin as the results of the drug were mostly not used for animal treatments.

Legesse *et al.* (2014) and Fentabil (2011) from food handlers, Alemu (2007) from cattle meat and Tesfaw *et al*. (2013) from milk reported 100% susceptibility of *Salmonella* isolates to gentamycin which almost comparable to the finding of this study, 97% (95%CI: 92.7-100). The slight difference might have been emanated from the fact that large numbers of isolates are being tested against gentamycin in recent study. However, the recent result showed higher susceptibility of isolates to gentamicin than 75% (Teshome and Anbessa, 2011) and 80% (Zelalem *et al*., 2011) from milk; 76.6% (Alao, *et al*., 2012) from faecal sample of cattle and 92.6% from human diarrheic patients (Ayalu *et al.,* 2011).

In current study nalidixic acid showed higher efficacy (60.6%; 95%CI: 48.5-72.7) than 20% efficacy report from Jimma (Ethiopia) (Teshome and Anbessa, 2012) and 10% from Kenya (Lakshmi *et al*., 2006) and lower efficacy than 100% efficacy report from Gondar (Ethiopia) (Legesse *et al.*, 2014), Nigeria (Smith *et al*., 2009) and India (Senthilkumar and Prabavaran, 2005). The variation in efficacy of nalidixic acid in these areas may be due to the frequency by which the drug is used.

Furthermore 75.8% (95%CI: 65.1-86.4) of the isolates were susceptible to sulfamethoxazole trimethoprim, which relatively conform to a report from Gondar (Ethiopia) (Legesse *et al*., 2014) and higher than susceptibility report from Ethiopia in different times (Zewdu and Cornelius, 2009). This drug was the most effective and 100% efficacy was reported for long time because this combination was not previously used in the country for the treatment of disease in cattle but recently reduction in efficacy might be related to the fact that the drug is being extensively used in human, poultry and cattle.

In recent study more than half (57.6%; 95%CI: 45.3- 69.8) of the isolates were resistant to second generation cephalosporins, Cefoxitin, used to be a common choice of treatment of salmonellosis. This shows that *Salmonella* antimicrobial resistance is posing a great challenge to medicine in general and public health in particular. Resistance to the broad spectrum cephalosporin is mainly due to the production of an enzyme called the extended spectrum β –lactamase. Widespread use of third generation cephalosporins is believed to be the major cause of the mutations in these enzymes that has led to the emergence of extended β –lactamases (Paterson and Bonomo, 2005).

Chloramphenicol, a drug with excellent blood brain barrier penetration ability, used to be an original indication for the treatment of typhoidal and non typhoidal salmonellosis, is now no longer prescribed as first line of treatment of because of universal presence of resistant *Salmonella* serotypes. In our finding it was also witnessed that 37.9% isolates were resistant to Chloramphenicol. The finding was in line with the 30% resistance report by Molla *et al*., (2003b) and 25% by Zelalem *et al.,* (2011) and Teshome and Anbessa, (2012). However it was lower than 62.3% resistance prevalence finding by Ayalu *et al.,* (2011) and 81.4% by Beyene (2008) from children. The difference between the findings could be due to the different in the distribution of resistant serotypes between humans and animals in that resistance reports from human is by far highest. Enzymatic inactivation by acetylation of the drug via different types of chloramphenicol acetyltransferases and phosphotrasferases, mutation of target sites and barriers are the mechanisms of chloramphenicol resistance and are due to incriminate use of the drug (Beyene, 2008).

37.9% of isolates were resistant to kanamycin, which is by far higher 0% resistance (100% susceptibility) report from chicken carcass at Addis Ababa (Molla *et al.,* 2003b) and 25% resistant report from Jimma (Teshome and Anbessa, 2012).

In looking the resistance pattern of commonly used antimicrobials, 60.6% (95%CI:48.5-72.7) of the *Salmonella* isolates were resistant to amoxicillin, which is relatively in agreement with 69.2% recent finding in Ethiopia (Legesse *et al*., 2014 and Nigeria (Okojie *et al.,* 2005). The resistant nature of *Salmonella* isolates to Amoxicillin might be ascribed to high level of utilization of this drug due to its relatively cheaper price and readily available nature to the local community in the current study area. This might create the opportunity for misuse of this drug thus heralding the emergence of resistant strains of *Salmonella.* Furthermore 56% of isolates were resistant to Ampicillin, This finding is in line with previous findings from Jimma and Addis Ababa that reported 54%, (Misganaw and David, 2010) and 59.4% (Zewdu and Cornelius, 2009) resistant isolates, in that order. But this ampicillin resistance pattern of *Salmonella* isolated during this study was lower than 100% resistance reported by Addis *et al*. (2011) from lactating cows and contact personnel in Addis Ababa and 82.3% resistance in pediatric patients in Addis Ababa. Furthermore; the current result was lower than 87.5%, 100%, and 91.8% of ampicillin resistant *Salmonella* isolates reported from Addis Ababa (Fentabil, 2011), Bahir Dar (Abera *et al.,* 2010) and Tamil Nadu (Valli *et al*., 2010), respectively. A worrying aspect of the current result is that it will also be difficult to treat other clinical infectious cases using this common antimicrobials commonly used for animals and humans.

Streptomycin alone or as a combination with other antimicrobials, was one of the most commonly used drugs both in humans and animals for long period of time and in recent study it was among the least effective antimicrobials with the resistance of 48.4% (95%CI: 36.1-60.9). This result is comparable with Alemu (2007) who reported resistance of 39.3% from slaughtered cattle. Higher than resistance report of 25% (Teshome and Anbessa, 2012), 24.1% (Sibhat, 2006) and 22.5% (Molla etal., 2003). Other investigators reported higher prevalence of streptomycin resistance than recent finding; 66.7%

(Zelalem *et al.*, 2011). The resistance to streptomycin in *Salmonella* isolates was reported in human in late 1970's (Sibhat, 2006). Both overuse, underuse through lack of access, inadequate dosing, poor adherence and substandard antimicrobials may play an important role in development of antimicrobial resistance (WHO, 2001).

Generally, in recent years, antimicrobial resistant *Salmonella* strains have been isolated with increasing frequency. Lack of stringent regulation and monitoring in the dispensing and use of antimicrobials in the veterinary establishments and mass inoculation of herds of animals by some farmers has risen as a contributory factor to increase antimicrobial resistance. The level of resistance is high to commonly used antimicrobials (Amocillin, ampicillin, streptomycin) in Ethiopia. Therefore attention against the rise in resistance of *Salmonellae* to antimicrobials is essential. Antimicrobial resistance is a global problem, which is not restricted to specific countries or bacterial pathogens. However, the problem of antimicrobial resistance is more complex and difficult in developing countries particularly in countries of sub-Saharan Africa like Ethiopia. This is mainly due to the fact that in most of these countries (i) Salmonella and other major pathogens including zoonotic bacteria are not routinely isolated and identified,(ii) the resistance of bacterial pathogens of veterinary and public health importance including salmonella to commonly used antimicrobials is rarely assessed either in public or animal health sector, (iii) people has easy access to varies antimicrobials and can purchase without prescription, and (iv) incomplete treatment courses due to patient non compliance are common practices (Molla, 2004; Sibhat, 2006; Alemu, 2007).

In conclusion, proportion of multidrug resistance has increased very high. Ciprofloxacin should be used as an empirical therapeutic agent. The potency of second and third generation cephalosporins have by far reduced. Therefore, alternative drug should be included in the essential drug list and prompt intervention measures should be taken so as to save potent drugs from resistance. Furthermore, large studies are needed on molecular pharmaco-epidemiology of *Salmonella.*

6. CONCLUSIONS AND RECOMMENDATIONS

In present study respective 9.8% and 7.92% animal level prevalence of *Salmonella* in apparently healthy slaughtered cattle and lactating cows in central Ethiopia was observed. The study also revealed 4.7% over all prevalence of *Salmonella* in dairy farms and 9.11% in abattoirs. *Salmonellae* were detected in abattoirs and farms from udder milk, tank milk, pooled tank swab, pooled buckets swab, pooled milkers' hand swab, feces from lactating cows, carcass swab, pooled butchers' hand swab, pooled butchers' knives swab, mesenteric lymph nodes, carcass hanging materials swab and cecal contents from slaughtered cattle. Carrier animals and contaminated tanks were found to be the main sources of milk contamination with *Salmonella* in farms while carcass hanging materials were the main sources for carcass contamination in the slaughtering process.

The majority of the *Salmonellae* isolates (84.8%) from the study were resistant to one or more of the tested antimicrobials. Of resistant isolates, 87.5% were found to be multi drug resistant. Based on the results and conclusions of this study, following recommendations are forwarded.

- Good hygienic practice in abattoirs and farms, specially carcass hanging materials and milk collecting tanks, should be put in place in order to eliminate food borne pathogens, including *Salmonella*
- Periodic surveillance of *Salmonella* carrier animals should be conducted in the farms; positive animals as well as their feces should be managed with precaution.
- Consumer awareness and proper cooking of meat and meat products and pasteurization of milk and milk products should be practiced to reduce the risk of salmonellosis and other food borne pathogens; and
- Prompt application of code of practice for antimicrobial used in veterinary medicine is very important and the use of resisted antimicrobials should be redirected.
- Further serotyping of *Salmonella* should be conducted

8. REFERENCES

AACA (Addis Ababa City Administration) (2004): Proclamation for establishment of "Kifle Ketema" and "Kebele" in Addis Ababa city, Addis Ababa, Ethiopia.

Abera, B., Biadegelgn, F. and Bezabih, B. (2010): Prevalence of *Salmonella* Typhi and intestinal parasites in food handlers in Bahir Dar town, northwest Ethiopia**.** *Ethiopian Journal of Health Development*, **24**(1)**:** 46-50.

Akinyemia, K.O., Smithb, S.I., Oyefolua, B.A.O. and Cokerc, A.O. (2005): Multidrug resistance in *Salmonella enterica* serovar Typhi isolated from patients with typhoid fever complication in Legos, Nigeria**.** *Public Health*, **119:** 321-327.

Alao, F., Kester, C., Gbagba, B. and Fakilide, F. (2012): Comparison of prevalence and antimicrobial sensitivity of *Salmonella* Typhimurium in apparently healthy cattle and goat in Sango-Ota, Nigeria. *International Journal of Microbiology*, **10**: 2

Alemayehu, D., Molla, B. and Muckle, A. (2003): Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle Ethiopia. *Tropical animal health and production*, **35**: 309-319.

Alemu, S. (2007): *Salmonella* serovars in apparently healthy slaughtered cattle at Bahir Dar abattoir, Ethiopia. MSc thesis, Addis Ababa University School of Graduate Studies.

Antonio, J. and Steele-Mortimer, O. (2009): *Salmonella*-the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Cell Microbiology*, **11**(11): 1579-1586.

Ayalu, A., Seyoum, B., Yimam, J., Adualem, G., Fiseha, S. and Vandeweerd, J.M. (2011): Antibiotic susceptibility pattern of *Salmonella* and *Shigella* isolates in Harar, eastern Ethiopia. *Journal of Infectious Diseases and Immunity*, **3**(8): 134-139.

Bada, R., Fofana, A. and Akakpo, A. (2006): Antimicrobial resistance of *Salmonella* isolated from poultry carcass in Dakar (Senegal). *Brazilian Journal of Microbiology*, **37**: 4

Beyene, B., Asrat, D., Mengistu, Y., Aseffa, A. and Wain, J. (2008): Typhoid fever in Ethiopia**.** *Journal of Infections in Developing Countries*, **2**(6)**:** 448-453.

Beyene, G. (2008): Phenotypic and molecular characterization of *Salmonella* species in Ethiopia. PhD thesis, Addis Ababa University School of Graduate Studies.

Blackburn, W.C. (1993): Review on rapid and alternative methods for detection of *Salmonella* in food. *Journal of applied bacteriology*, **7**: 316-325.

Boyle, E. C., Bishop, J. L., Grassl, G. A. and Finlay, B. B. (2007): *Salmonella*: from Pathogenesis to Therapeutics. *ASM Journal of Bacteriology*, **189**: 1489-1495.

Brandt, R. (2012): Functional and phenotypic profiling of innate immunity during *Salmonella* infection. PhD thesis, Technical University of Denmark.

Brenner, F.W., Villar, R.G., Anjulo, F.G., Tauxe, R. and Swaminathan, B. (2000): *Salmonella* nomenclature. *Journal of Clinical Microbiology*, **38**(7): 2465-2467.

Broz, P., Newton, M., Lamkanfi, S., Mariathasan, V., Dixit, M. and Monack, M. (2010): Redundant roles for inflammation, some receptors NLRP3 and NLRC4 in host defense against *Salmonella*. *Journal of Experimental Medicine*, **207**: 1745-55.

Busani, L., Cigliano, A., Taioli, E., Caliuri, V., Chiavacci, L., Dibella, C., Battisti, *et al*. (2005): Prevalence of *Salmonella enterica* and *Listeria monocytogenes* contamination in food of animal origin in Italy. *Journal of Food Protection*, **68**: 1729 - 1733.

Buzby, J and Roberts, T. (2009): "The Economics of Enteric Infections: human food borne disease costs. *Gastroenterology*, **136** (6): 1851-62.

CDC (Center for Disease control and Prevention) (2004): Multi-drug *Salmonella* Typhimurium infection from milk contaminated after pasteurization. http://wwwnc.cdc.gov/eid/article/10/5/03-0484

CDC (Center for Disease control and Prevention) (2011): National *Salmonella* Surveillance overview, Atlanta, Georgia: US Department of Health and Human Service. http://www.cdc.gov/ncezid/dfwed/

CDC (Center for Disease control and Prevention) (2013): National *Salmonella* Surveillance overview, Atlanta, Georgia: US Department of Health and Human Service. http://www.cdc.gov/ncezid/dfwed/

CLSI (Clinical and Laboratory Standards Institute) (2012): Performance standards for antimicrobial susceptibility testing.17thInformational Supplement CLSI Document M100-S**17** 27

CSA (Central statistical Agency) (2007): Population and Housing Census of Ethiopia. p. 22. Retrieved 2011-12-02

Charles, S.C., Nagaraj, K.V. and Sivanandan,V.A.(2000): lipid conjugated immunostimulating complex subunit vaccine against *Salmonella* infection in turkeys. *Avain Disease*, **37**: 477-484.

Chiu, C., Su, L. and Chu, C. (2004): *Salmonella enterica* serotype Choleraesuis: epidemiology, pathogenesis, clinical disease and treatment. *Clinical Microbiology Reviews*, **17**: 311-322.

Coetzer, J, W., Thomson, G.R., Tustin, R.C. and Kriek, N.P.J. (1994): Infectious diseases of Livestock with special reference to South Africa. Oxford Newyork, Pp. 1100-1112.

D'Aoust, J. (1997): *Salmonella.* In: Doyle, M.P., Beuchat, L.R. and Montville, T.J. (eds): Food microbiology-fundamentals and fronteries. ASM press, Washington D.C., Pp. 129-158.

D'Aoust, J.Y. (2000): *Salmonella:* In: Lund, B.M., Baird-Parker, T.C. and Gould, G.W. (eds): The microbiological safety and quality of food. Aspen Publishers, Mary land, Pp. 1233-1279.

D'Aoust, J.Y. (2001): *Salmonella*. In: Ronald, G.L. and Santos, G. (eds): Guide to food borne pathogens. A John Wiley and Sons Inc.Publication, New York, Pp. 163-191

Dabassa, A. and Bacha, K. (2012): The prevalence and antibiogram of *Salmonella* and *Shigella* isolated from abattoir, Jimma town, south west Ethiopia. *International Journal of Pharmaceutical and Biological researches,* **3**: 143-148.

Denise, M., Anne, M. and Stanley, F. (2004): Persistent bacterial infections: the interface of the pathogen and the host immune system. *Nat Rev Microbiol*, **2**: 747–765.

Ejeta, G., Molla, B., Alemayehu, D. and Muckle, A. (2004): *Salmonella* serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Reveu de Medecine Veterinaire*, **155** (11): 547- 551.

Fentabil, G. (2011): Isolation of *Salmonella* species among apparently healthy food handlers of Addis Ababa University students' cafeteria, Addis Ababa, Ethiopia. MSc thesis, Addis Ababa University School of graduate studies.

Forough, T., Elahe, T., Manochehr, M., Ebrahim, R. and Rafie, S. (2012): Occurrence and antibiotic resistance of *Salmonella* spp isolated from raw Cow's milk from Shahahrekord, Iran. *International Journal of Microbiological Research,* **3**(3): 242-245.

Girmay, G. (2013): Evaluating the relative resistance of different poultry breeds to *Salmonella* Typhimurium. MSc thesis, Addis Ababa University College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia.

Gray, T. and Fedorka-Cray, J. (2002): "*Salmonella*." In: Dean, O. and Riemann, P. (eds): Foodborne Diseases. Academic Press, San Francisco, Pp. 55 - 68.

Grimont, P.A.D. and Weill, F. (2007): Antigenic formulae of the *Salmonella* serovars, WHO Collaborating Centre for Reference and Research on *Salmonella*. Paris: Pasteur institute http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089.

Guma, M., Ronacher, L., Liu-Bryan, R., Takai, S., Karin, M. and Corr, M. (2009): Caspase 1-independent activation of interleukin-1beta in neutrophil-predominant inflammation. *Arthritis Rheum.,* **60**: 3642 - 50.

Halderman, M. (2004): The policy economy of pro-poor livestock policy-marketing in Ethiopia. FAO, PPLPI Working Paper No. 19, http://www.fao.org/ag/pplpi.html

Hendriksen, R.S., Veira, Ar., Karlsmose, S., LoFoWong, D.M., Wegener, H.C. and Aerestrup, FM. (2011): Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne pathogene disease*, **8**(8): 887-900.

Heuzenroeder, M.W., Murray, C.J., Davos, D. and Ross, I.L. (2004): *Salmonella* typing and colonization of chicken by characterized *Salmonella* Sofia. Rural industries Research and Development Corporation. RIRDC publication No 04/138 RIRDC project No IMV-3A

Hitoshi, I. (2006): Effect of radiation decontamination on drug resistant bacteria. *Journal of Food Irradiation,* **41**: 9-13.

http://www.news-medical.net/health/Salmonella-History.aspx,salmonellahistory, accessed June 4, 2014.

http://en.wikipedia.org/wiki/Asella. september, 2013

http://www.vetmed.wisc.edu/pbs/zoonoses/gik9fel/salmonella.html

Ibrahim, M.A., Emeash, H.H., Ghoneim, H. and Abdel-Halim, H. (2013): Sero-epidemiological Studies on Poultry Salmonellosis and its Public Health Importance. *Journal of World's Poultry Research*, **3**(1): 18-23.

ILCA (International Livestock Centre for Africa) (1994): Annual Program Report, Addis Ababa, Ethiopia.

Isaacson, R.E., Firkins, L.D., Weigel, R.M., Zuckermann, F.A. and Dipietro, J.A. (1999): Effect of transportation and feed withdrawal on shedding of *Salmonella* Typhimurium among experimentally infected pigs. *American Journal of Veterinary Researches*, **60**: 1155 - 1158.

ISO (International Organization for Standardization) (1998): Microbiology of food and animal feeding staff-horizontal method for the detection of *Salmonella*, ISO, 6579 Geneva.

ISO (International Organization for Standardization) (2002): Microbiology of food and animal feeding staff-horizontal method for the detection of *Salmonella*, 4th ed., ISO, 6579 Geneva.

Izzo, M., Mohler, V. and House, J. (2011): Antimicrobial susceptibility of *Salmonella* isolates recovered from calves with diarrhea in Australia**.** *Australian Veterinary Journal*, **89**(10): 402 - 8.

Jakabi, M., Buzzo, A.A., Ristori, C.A., Tavechio, A.T., Sakuma, H., Paula, A.M., and Gellis, D.S. (1999): Observacoes laboratoriais sobre surtos alimentares de *Salmonella* spp.ocorridos na Grande sao Paulo, no period de 1994 a 1997. *Revista do Instituto adolfo Lutz.,* **58**: 47-51

Jay, J.M. (2000): Modern food microbiology. 6th ed., Aspen publication, Maryland, Pp. 511-525.

Jones, W., Watson, R., Wallis, and S. (2004): Salmonellosis. In: Andrew, H., Blowey, W., Boyd, H., and Eddy, G. (eds): Bovine Medicine: Disease and Husbandry of Cattle, 2nd ed., Blackwell science LTD. UK. Pp. 215-230.

KARC (Kulumsa Agricultural Research Center) (2008): Annual Report of KARC, Asella, Ethiopia. p. 6.

Karoline, M. (2012): Genetic and phenotypic characteristics of importance for clonal success and diversity in *Salmonella* National Food Institute Technical University of Denmark, PhD thesis.

Kaur, J. and Jain, S. (2011): Role of antigens and virulence factors of *Salmonella enterica* serovar Typhi in its pathogenesis. *Microbiological research*, **167**(4): 199-210.

Lee, H., Kim, S., Kim, K., Mo, I., Woo, Y., Kwon, Y. and Kim, T. (2000): Immunogenicity of outer membrane protein extracted from *Salmonella* Gallinarum in chickens. *National Veterinary Research Institute,* **37**: 555-568.

Legesse, G., Nishanwork, W. and Amsalu, F. (2014): Identification of drug-resistance *Salmonella* from food handlers at the University of Gondar, Ethiopia. *BMC Research Notes*, **7**: 545

Libby, J., Halsey, A., Altier, C., Potter, J. and Gyles, L. (2004): *Salmonella*. In: Gyles, L., Prescott, F., Snoger, G. and Theon, O. (eds). Pathogenesis of bacterial infections in animals, 3rd ed. Blackwell publishing, USA, Pp. 143-167.

Luciana, F., Tatiana, A., Renee, M., Andrease, J. and Renato, L. (2012): Salmonellosis in cattle: Advantages of being an experimental model. *Research in Veterinary Science*, **93**(1): 1-6.

Maddox, C.W. (2003): *Salmonella* detection methods. In: Torrence, M.E. and Isaacson, R. (eds): Microbial food safety in animal agriculture. Current topics. Iowa state press, Black well publishing company, Pp. 83-88.

Magiorakos, AP., Srinivasan, A., Carey, R.B., Carmeli,Y., Falagas, M.E., Giske, C.G., Harbarth *et al*. (2012): Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiological Infection*, **18(**3): 268 - 81

Mahami, T., Odonkor, S., Yaro, M. and Adu-Gyamfi, A. (2011): Prevalence of antibiotic resistant bacteria in milk sold Accra. *International Research Journal of Microbiology*, **2**: 126-132.

McEnvoy, J., Doherty, A., Sherdan, J. and McDowell, D. (2003): The prevalence of *Salmonella* spp. in bovine fecal, rumen and carcass samples in commercial abattoir. *Journal of Applied Microbiology*, **94**: 693-700

McGhie, E., Brawn, L., Hume, P., Humphreys, D. and Koronasks, V. (2009): *Salmonella* takes control; effectors driven manipulation of the host. *Current opinion microbiology*, **12**: 117-124.

Misganaw, B and David, W. (2013): A study of *Salmonella* carriage amongsymptomatic food-handlers in southern Ethiopia. *International Journal of Nutrition and Food Sci*ence, **2**(5)**:** 243 - 245.

MØlbak, K., John, E. and Henrik, C. (2006): *Salmonella* infections. In: Foodborne infections and intoxications, Elsevier Inc., Pp. 57-114.

Molla, B., Alemayehu, D. and Salah, W. (2003a): Source and distribution of *Salmonella* serotypes isolated from food animals, slaughter house personnel and retail meat products in Ethiopia: 1997- 2000. *Ethiopian Journal of health development*, **17**: 63-70.

Molla, B., Mesfin, A. and Alemayehu, D. (2003b): Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. *Ethiopian Journal of Health Development*, **17**(2): 131-149.

Molla, W. (2004): Cross sectional study on *Salmonella* in apparently healthy slaughtered sheep and goat at Addis Ababa and Modjo abattoirs. MSc thesis, Addis Ababa University Faculty of Veterinary Medicine, Debrezeit, Ethiopia.

Molla, W., Molla, B., Alemayehu, D., Muckle, A., Cole, L. and Wilkie, E. (2006): Occurrence and antimicrobial resistance of *Salmonella* serovars in apparently healthy slaughtered sheep and goats of central Ethiopia**.** *Tropical Animal Health Production*, **38:** 455-62.

Mortimer, C., Peters, T., Gharbia, S., Logan, J. and Arnold, C. (2004): Towards the development of a DNA-sequence based serotyping of *Salmonella*. *Biomedical central microbiology*, **4**: 1982 - 1992.

Muluneh, G. and Kibret, M. (2015): *Salmonella* spp. and risk factors for the contamination of slaughtered cattle carcass from a slaughterhouse of Bahir Dar Town, Ethiopia. *Asian Pacific Journal of tropical diseases*, **5**(2): 130-135.

Murphy, S. and Boor, K. (2010): Sources and causes of high bacterial count in raw milk: An abbreviated review.

Nielsen, T.D., Kudahl, A.B., Østergaard, S. and Nielsen, L.R. (2013): Gross margin losses due to *Salmonella* Dublin infection in Danish dairy cattle herds estimated by simulation modelling. *Preventive Veterinary Medicine,* **111**: 1-2.

NMSA (National Meteorology Service Agency) (2006): Monthly Climate Bulletin, Adama Branch, Adama, Ethiopia, p. 7.

Nouichi, S. and Hamdi, T. M. (2009). Superficial Bacterial Contamination of Ovine and Bovine Carcasses at El-Harrach Slaughterhouse (Algeria). E*uropean Journal of. Science Research*, **38**: 474 - 485

Nyeleti, C., Hildebrandt, G., Kleer, J. and Molla, B. (2000): The prevalence of *Salmonella* in Ethiopian cattle and minced beef. *Berliner und Munchener Tierarztliche Wochenschrift,* **113**: 131-134.

OIE (Office International des Epizooties) (2005): Salmonellosis: Paratyphoid, non-typhoidal salmonellosis. Institute for international cooperation in animal biogenetics. An OIE collaborating center for food security and public health, Iowa State University, College of Veterinary Medicine. http:/www.cfsph.iastate.ed.

Okojie, O.H., Wagbatsoma, V.A. and Ighoroge, A. D. (2005): An assessment of food hygiene among food handlers in a Nigerian University campus**.** *Nigerian Post-graduate Medical Journal*, **12**(2)**:** 93-96

Olsen, J.E., Brown, D.J., Skov, M.N. and Christensen, J. (1993): Bacteria typing methods suitable for epidemiological analysis: application in investigation of salmonellosis among livestock. *Veterinary Quarterly*, **15**: 125-135.

Padungtod, P and Kaneene, B.J. (2006): *Salmonella* in food animals and humans in northern Thiland. *International Journal of food microbiology*, **108**: 346-354.

Paterson, D. L. and Bonomo, R. A. (2005): Extended-spectrum beta-lactamases: a clinical update. *Clinical Microbiology Review*, **18**: 657-86.

Quinn, P.J., Carter, M. E., Markey, B. and Carter, G.R. (1994): Bacterial pathogens: microscopy culture and identification. In: Clinicall veterinary microbiology. Wolf publishing, Londo, Pp. 21-60.

Quinn, P.J., Carter, M. E., Markey, B. and Carter, G.R. (1999): *Enterobacteriaceae*. In: Clinical veterinary microbiology. Molbsy international limited, London, Pp. 226-236.

Radostitis, O.M., Blood, D.C. and Gray,C.C. (1994): Veterinary Medicine, a Text Book of the Disease of Cattle, Sheep, Goats and Horses. 8 th ed. London.Ballier Tidal, Pp. 730 - 747.

Randall, J. B. (2001): Salmonellosis in Cattle. Colorado serum campany report. **1(**8)

Scharffe, L. (2012): Economic burden from health Losses due to foodborne illness in the United States. *Journal of Food Protection*, **75**(1): 123–131.

Samuel, J-L., O'Boyle, D.A., Mathers, W.J. and Frost, A.J. (1980): Distribution of *Salmonella* in Carcass of normal cattle at slaughter. *Research in veterinary science,* **28**: 368 - 372.

Sara, E., Guy, H., Kendra, A., Dayna, A., Henry, R., Jacob, R., Lyda *et al*. (2013): Substantial within-animal diversity of *Salmonella* isolates from lymph nodes, feces, and hides of cattle at slaughter. *Applied Enviromental Microbiology,* **79** (15): 4744 – 4750

Schmidt, H. and Hensel, M. (2004): Pathogenicity islands in bacterial pathogenesis. *Clinical Microbiology Review,* **17**: 14–56.

Seifert, H.S.H. (1996): Tropical animal health. Kluwer Acadamic Publisher, Dorotrecht. Pp. 368 - 371.

Senthilkumar, B. and Prabavaran, G. (2005): Multidrug resistant *Salmonella* Typhi in asymptomatic Typhoid carriers among food handlers in Mamakkal District, Tamil nadu, Indian**.** *Journal of Medical Microbiology*, **23**(2)**:** 92-94.

Sibhat, B. (2006): Prevalence, distribution and antimicrobial resistance of *Salmonella* isolates from slaughtered cattle in Debrezet, Ethiopia. MSc thesis, Addis Ababa University Faculty of Veterinary Medicine

Singh, B.R. (2009): *Salmonella* vaccines for animals and birds and their future Perspective. *The Open Vaccine Journal*, **2**: 100-112.

Smith, S.I., Bamidele, M., Goodluck, H.A., Rowora, M.N., Omonigbehn, E.A. and Opere, B.O. (2009): Antimicrobial susceptibilities of *Salmonellae* isolated from

foodhandlers and cattle in Lagos, Nigeria**.** *International Journal of Health Researches*, **2:** 189 - 193.

Steele**,** M.L., McNab, W.B., Poppe, C., Griffiths, M.W., Chen, S., Degrandis, S.A. Fruhner *et al*. (1997): Survey of Ontario bulk tank milk for foodborne pathogens. *Journal of Food Protection*, **60**: 1341-1346.

Teklu, A. (2008): Prevalence and serotype distribution of *Salmonella* in slaughtered sheep and goat and abattoir environment in an export abattoir, Modjo, Ethiopia. MSc thesis, Addis Ababa University Faculty of Veterinary Medicine, Debrezeit, Ethiopia.

Tesfaw, L., Taye, B., Alemu, S., Alemayehu, H., Sisay, Z. and Nigussie, H. (2013): Prevalence and antimicrobial resistance profile of Salmonella isolates from dairy products in Addis Ababa, Ethiopia. *Global Journal of Industrial Microbiology*, **1**(1):023 - 027.

Teshome, T. and Anbessa, D. (2012): Prevalence and antimicrobial resistance of *Salmonella* isolated from raw milk samples collected from Kersa district, Jimma zone, southwest Ethiopia. *Journal of Medical Sciences,* **12**: 224-228.

Thrustfield, M. (2005): Veterinary epidemiology. 3rd ed., Blackwell sciences Ltd., London, Pp. 228 - 246.

Tibaijuka, B., Molla, B., Hildebrandt, G., Kleer, J. and Salah, W. (2002): Antimicrobial resistance to *Salmonellae* isolated from retail raw chicken meat and giblets in Ethiopia. *Bulletin of Animal Health and Production in Africa,* **50**(2): 86 - 95

Tierrez, A. and Garcia-del Portillo, F. (2005): New concepts in *Salmonella* virulence: the importance of reducing the intracellular growth rate in the host. *Cell Microbiology*, **7**: 901– 909.

Valli, A., Selvan, N., Sudha, A., Dhananjeyan, V. and Iyappan, P. (2010): Screening of asymptomatic typhoid carriers from nail samples from roadside foodhandlers in Tamil Nadu, India**.** *Current Research of Bacteriology,* **3:** 238-244.

Venter, B.J., Myburgh, J.G. and Van der, W. (1994): Bovine *Salmonellosis.* In: Coetzer, J.W., Thomson, G.R. and Tustin, R.C.(eds). Infectious diseases of Live stock with special reference to southern Africa. Oxford university press, Pp. 1104-1112.

Viera-Pinto, M., Temudo, P. and Martins, C. (2005): Occurrence of *Salmonella* in ilium, ileocolonic lymphnodes, tonsils, mandibular lymph nodes and carcasses of pigs slaughtered for consumption. *Journal of veterinary Medicine Series B,* **52**: 476-481

WHO (World Health Organization) (1988): Salmonellosis control: Role of animal and product hygien. Report of a WHO Expert Committee. Geneva: WHO technical report series, No.774.

WHO (World Health Organization) (2007): Initiative to estimate the global burden of food borne diseases. First formal meeting of the food borne diseases burden epidemiologyreferencegroup(FERG).http://www.who.int/foodsafety/publications/foodborne_disease/FERG_Nov07.pdf.

WHO (2001): WHO global strategy for containment of antimicrobial resistance. WHO, Geneva, Switzerland, Pp. 1-92.

Wigley, P., Berchien, A., Page, L., Smith, L. and Barrow, A. (2001): *Salmonella enterica* serovar Pollorum persists in splenic macrophages and in reproductive tract during persistent, disease free carriage in chickens. *Infectious immunology,* **69**: 7873-7879.

Woldemariam, E., Molla, B., Alemayehu, D. and Muckle, A. (2005): Prevalence and distribution of *Salmonella* in apparently healthy slaughterd sheep and goats in Debrezeit, Ethiopia. *Small ruminant research*, **58:** 19-24.

Woodward, M.J., Gettinby, C., Breshlin, M.F., Corkish, J.D. and Houghton, S. (2002): The efficacy of salenvac, a *Salmonella enteric* subsp. *enterica* serotype Enteritidis iron restricted bacterin vaccine, in laying chickens. *Avian Pathology,* **31**: 383 - 392.

Wray, C. and Davies, R.H. (2003): The epidemiology and ecology of *Salmonella* in meat producing animals. In: Torrence, M.E., and Isaacson, R.E. (eds). Microbial food safety in animal agriculture. Current topics 1st ed USA, Blackwell Publishing. Pp.73-82.

Zelalem, A., Nigatu, K., Zufan, S., Haile, A., Alehegne, W. and Tesfu, K. (2011): Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating

cows in contact humans in dairy farms of Addis Ababa: A cross sectional study. *BMC Infectious Diseases*, **11**: 222

Zewdu, E. (2004): Prevalence, distribution and antimicrobial resistance profile of *Salmonella* isolated from food items and personnel in Addis Ababa, Ethiopia. MSC thesis, Addis Ababa University Faculty of Veterinary Medicine, Debrezeit, Ethiopia.

Zewudu, E. and Cornelius, P. (2009): Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa. *Ethiopian Tropical Animal Health and Production*, **41**(2)**:** 241-249

9. APPENDICES

Appendix I: Media used and preparations for isolation and identification of *Salmonella*

1. Buffered peptone water ISO 6579, ISO 22964, ISO 6887, ISO 19250

Composition (g/litres): casein pancreatic digest 10; sodium chloride 5.0; disodium phosphate 3.3; monopotassium phosphate 1.5; equivalent to 9 grams of disodium hydrogen phosphate deodecarbohydrate; pH= 7 ± 0.2 at 25^{O}C.

Preparation; suspend 20 grams of the medium in one liters of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in to appropriate container and sterilize in autoclave at 121^{O}C for 15 minutes.

2. Rappaport vassiliads *Salmonella* enrichment broth (Himedia laboratories Pvt.Ltd, Mumbai, india):

Compositions (g/litres): soya peptone 4.5; sodium chloride 8.00; potassium di-hydrogene phosphate 0.6; dipotasium phosphate 0.4; magnesium chloride hexahydrate 29.00; malachite green 0.036; pH after sterilization 5.2 ± 0.2 at 25^{O}C.

Preparation; suspend 27.11 grams of dehydrated medium in 1000ml purified /distilled water heat if necessary to dissolve the medium completely. Dispense as desired in to tubes and sterilize by autoclaving at 115^{o}C for 15 minutes.

5. Selenite broth (selenite F Broth) (Twin pack) Part A, and Selelnite broth (selenite F broth) part B. (Himedia laboratories Pvt.Ltd, Mumbai, india):

Composition of part A (g/litres): casein enzaymatic hydrolysate,5; lactose,4.0; sodium phosphate, 10; pH = 7.0 ± 0.2 at 25 ^{O}C.

Composition of Part B; sodium hydrogen selenite; 4.0

Preparation; suspend 4 grams of part B in 1000ml distilled water. Add 19.0 grams of part A.mix well. Warm to dissolve the medium completely. Distribute in sterile test tubes. Sterilize in boiling water bath or a free flowing steam for 10 minutes. Do not autoclave. Excessive heating is determinate. Discard the prepared media if large amount of selenite is reduced (indicated by red precipitate at the bottom of the tubes).

6. Xylose-lysine Deoxycholate agar (XLD) ((Himedia laboratories Pvt.Ltd, Mumbai, india):

Compositions (g/litres): yeast extract 3.00; L-lysine 5.00; Lactose 7.500; sucrose 7.500; xylose 3.500; sodium chloride 5.00; sodium deoxycholate 2.5; sodium thiosulfate 6.8; ferric ammonium citrate 0.800 phenol red 0.08; agar 15; pH= 7.4±0.2

Preparations; susped 56.68 grams in 1000ml of distilled water heat with frequent agitation until the medium boils. Do not autoclave or over heat. Transfer immediately to a water bath at 50^{o}C. After cooling pour in to sterile petri plates. It is advisable not to prepare large volume which will require prolonged heating.

7. S.S. agar (*Salmonella*, *Shigella* agar) (Oxoid LTD, Basingstoke, Hampshire, England):

Composition: Lab-Lemco powder 5.0; peptone 5.00; lactose 10; bile salt salt 8.5; sodium citrate 10; sodium thiosulfate 8.5; ferric citrate 10; brilliant green 0.00033; neutral red 0.025; agar 15.0

Preparations; suspend 63 gram in 1L of distilled water. Bring to the boil with frequent agitation, and allow simmering gently to dissolve the agar. Do not autoclave. Cool to about 50^{o} C , mix and pour in to petri dishes..

8. Nutrient agar (Himedia laboratories Pvt.Ltd, Mumbai, india):

Composition (g/litres): peptic digest of animal tissue 5; sodium chloride 5; beef extract 1.5; yeast extract 1.5; agar 15; pH=7.4±0.2 at 25 ^{O}C.

Preparations: suspend 28.0 grams in 1000ml distilled water. Heat to boil to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure at (121 ^{o}C) for 15 minutes. If desired the medium can be enriched with 5-10% blood or other biological fluids. Mix well and pour in to sterile Petri plates.

9. Triple Sugar Iron Agar (Oxoid LTD, Basingstoke, Hampshire, England):

Compositions (g/litres): 'Lab-Lemco' powder 3.0; yeast extract 3; peptone 20; sodium chloride 5; lactose 10; sucrose 10; glucose 1; ferric citrate 0.3; sodium thiosulphate 0.3; phenol red 0.024; agar 12; pH=7.4±0.2 at 25 ^{O}C.

Preparations; suspend 65 g in 1 liter of distilled water. Bring to the boil to dissolve completely. Mix well and distribute to the containers. Sterilize by autoclaving at 121 ^{O}C for 15 minutes. Allow to set as slopes with 2.5 cm butts.

10. MR- VP Medium (Buffered glucose broth) (glucose phosphate broth) (Himedia laboratories Pvt.Ltd, Mumbai, india):

Compositions (g/litres): buffered peptone 7; dextrose 5; dipotassium phosphate 5; pH=6.9 ± 0.2 at 25 ^{O}C.

Preparations; suspend 17 g in 1000ml distilled water. Heat if necessary to dissolve the medium completely, distribute in test tubes in 10 ml amount and sterilize by autoclaving at 15 Ibs pressure at (121 ^{o}C) for 15 minutes.

11. Mueller Hinton Agar (Oxoid LTD, BASINGSTOKE, hampshire, England): Compositions (g/litres): beef dehydrate infusion from 300; casein hydrolysate 17.5; starch 1.5; agar; 17.0; pH=7.3 ± 0.1 at 25 ^{O}C.

Preparations; suspend 38 g in 1 litre of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 121^{0}C for 15 minute.

12. Tryptose soya broth (Oxoid LTD, BASINGSTOKE, hampshire, England):

Compositions (g/litres): pancreatic digest casein 17; enzymatic digest of soya bean 3; sodium chloride 5; Di-potassiun hydrogen phosphate 2.5; glucose 2.5; pH= 7.3 ± 0.2 at 25 ^{O}C.

Preparations; dissolve 30 g in 1 liter of water (purifies, as required) an distribute in to final containers. Sterilize by autoclaving at 121^{o}C for 15 minutes.

13. Simmon citrate agar (Himedia laboratories Pvt.Ltd, Mumbai, india):

Compositions (g/litres): magnesium sulphate 0.2; ammonium dihydrogene phosphate 1; dipotassium phosphate 1; sodium citrate 2; sodium chloride 5; bromothymol blue 0.08; agar 15; pH= 7.3 ± 0.2 at 25 ^{O}C.

Preparations; suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired in tubes or flasks. Sterilize by autoclaving at 15 Ibs pressure at (121 ^{o}C) for 15 minutes.

14. Urea agar base(Christensen)(Autoclavable): (Himedia laboratories Pvt.Ltd, Mumbai, india):

Composition(g/litres):peptic digest of animal tissue, 1.00; dextrose, 1.00; sodium chloride, 5.00; disodium phosphate, 1.20; mono potassium phosphate, 0.8; phenol red, 0.012 agar; 15.pH=6.8 ± 0.2 at 25 ^{O}C

Preparation; suspend 24 gram in 950 ml distilled water. Heat to boil to dissolve the medium completely. Sterilize by autoclaving at 10lbs pressure (115^{o}C) for 20 minutescool to 50^{o}C and ascetically add 40% urea solution. (FDo48) and mix well. Dispense into sterile tubes and allow setting in the slanting position. Do not over heat or reheat the mediau as urea decomposes very easily.

13. Lysine iron agar (LIA) (DifcoTM), BectonDickson, Claix, France):

Composition (G/l): peptone, 5.0; yeast extract, 3.0; dextrose, 1.0; L-lysine HCL, 10.0; ferric ammonium citrate, 0.5; sodium thiosulphate, 0.04; bromocresol purple, 0.02; agar 15

Preparation; suspend 34.5 gram of the powder in 1 liter of distilled water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute completely dissolve the powder. Autoclave at 121^{o}C for 12 minutes.

Reagents required for Voges-Proskaur(VP) reaction

1. 1-Naphihol, ethanolic solution.

Composition; 1-Naphihol 6gm; ethanol, 95%(volume fraction) 100ml

Preparation; dissolve the 1-naphihol in the ethanol.

2. Potassium hydroxide solution

Compositon; potassium hydroxide 40gm; water; 100ml

Preparation; dissolve the potassium hydroxide in the water.

Reagent required for indole test

1. Kovac's reagent(UNI_CHEM) ready made.

   Composition: 4-dimethyl aminobenzaldehyde 5gm; ethanol alcohol75ml; hydrochloric acid 25ml

Appendix II: Preparation of 0.5 McFarland turbidity standards.

Solution A (0. 48 M $BaCl_2 2H_2O$)

1,172g $BaCl_2 2H_2O$

make up to 100ml with distilled water

Solution B (0.18M H_2SO_4)

1ml H_2SO_4) (analar grade, sp.gr.1.84)

make up to 100ml with didtilled water

For standard:

Add 0.5 ml solution A to 95.5 ml of solution B. shake vigorously and dispense in to 4-5ml sealed screw capped vials and store at room temperature in the dark place.

Appendix III: work sheet for recording colony morphology and biochemical reactions of presumptive salmonella isolates

Sample no.	Sample type	Spp	Date of collection	Origin	Total slaughter volume	Colony characteron		TSI				LIA			Urease	VP	MR	Indol	cittrate
						XLD agar	SS agar	Lac/Suc	Gluc	H2S	Gas	Butt	Slant	H2S					