ADDID ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

PREVALENCE, DISTRIBUTION AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* ISOLATES FROM SLAUGHTERED CATTLE IN DEBRE ZEIT, ETHIOPIA

By
BERHANU SIBHAT KASSA

JUNE 2006
DEBRE ZEIT, ETHIOPIA

TABLE OF CONTENTS

PAGE

LIST OF TABLES ..... **v**

LIST OF APPENDICES ..... **v**

LIST OF ABBREVIATIONS ..... **vi**

ACKNOWLEDGEMENS ..... **viii**

ABSTRACT ..... **ix**

1. INTRODUCTION **1**

2. LITRATURE REVIEW **4**

2.1. Taxonomy and nomenclature **4**

2.2. Detection of *Salmonella* **5**

2.2.1. Pre-enrichment in non-selective liquid medium 6

2.2.2. Enrichment in selective liquid media 6

2.2.3. Plating out and identification 7

2.2.4. Confirmation 9

2.2.4.1. Biochemical confirmation 9

2.2.4.2. Serological confirmation 10

2.2.5. Typing of *Salmonella* 11

2.2.5.1. Serotyping 11

2.2.5.2. Phage typing 11

2.3. Epidemiology **12**

2.3.1. Distribution 12

2.3.2. Host range 14

2.3.3. Sources of infection and transmission 16

3.3.4. Carrier state 18

2.4. Clinical picture **19**

2.4.1. In cattle 19

2.4.2. In humans 21

2.5. Treatment and control 22

2.5.1. Treatment 22

2.5.2. Control and prophylaxis 23

2.6. Antimicrobial resistance of *Salmonella* 24

2.7. *Salmonella* in Ethiopia 26

2.7.1. *Salmonella* serotypes 26

2.7.2. Antimicrobial resistance 27

3. MATERIALS AND METHODS 30

3.1. Study area 30

3.2. Study animals 31

3.3 Study design 31

3.4. Sampling 32

3.4.1. Sampling procedures 32

3.4.2. Sample processing 34

3.5. Isolation and identification of *Salmonella* 35

3.5.1. Pre-enrichment 35

3.5.2. Selective enrichment 35

3.5.3. Selective plating and identification 35

3.5.4. Biochemical identification 36

3.5.5. *Salmonella* serotyping 37

3.5.6. Resistance to antimicrobial agents 37

4. RESULTS 39

4.1. Prevalence of *Salmonella* 39

4.2. Distribution of serotypes 41

4.3. Antimicrobial resistance 43

5. DISCUSSION 47

5.1. Prevalence **47**

5.2. Serotypes **50**

5.3. Antimicrobial resistance **53**

6. CONCLUSION AND RECOMMENDATIONS **59**

7. REFERENCES **61**

8. APPENDICES **78**

9. CURRICULUM VITAE **83**

10. SIGNED DECLARATION SHEET **85**

LIST OF TABLES

Table 1. Diseases caused by *Salmonella* subspecies I serotypes in humans and higher vertebrates 15
Table 2. Status and pattern of *Salmonella* antimicrobial resistance in humans in Ethiopia 28
Table 3. Status and pattern of *Salmonella* drug resistance in animals and animal products in Ethiopia 29
Table 4. Type and number of samples collected from slaughterhouse at Debre Zeit 34
Table 5. Antimicrobials and concentrations used to test susceptibility of *Salmonella* isolates 38
Table 6. Prevalence of *Salmonella* from different samples from slaughter cattle and environment 40
Table 7. Distribution of *Salmonella* serotypes by sample type 41
Table 8. Serotype distribution in samples of carcass positive animals 42
Table 9. Multiple serotypes isolated from different samples of individual slaughter cattle in Debre Zeit 43
Table 10. Antimicrobial resistance patterns of *Salmonella* isolated from slaughtered cattle at Debre Zeit 44
Table 11. Distribution of antimicrobial resistance by sample source 46

LIST OF APPENDICES

Appendix 1: Media used and preparations for the isolation and identification of *Salmonella* 78
Appendix 2: Preparation of 0.5 McFarland Turbidity Standard. 81
Appendix 3: Work sheet for recording colony morphology and biochemical reactions of presumptive *Salmonella* isolates 82

LIST OF ABBREVIATIONS

ABC	α β- chromogenic medium
ACSSuT	Ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline
AFSSA	Agence Française de Securite Sanitaire des Aliments
AIDS	Acquired immunodeficiency syndrome
BG	Brilliant green agar
BPLS	Brilliant green phenol red lactose sucrose agar
BPW	Buffered peptone water
BS	Bismuth sulphite
CC	Caecal content
CDC	Centre for Disease Control and Prevention
CI	Confidence interval
Defra	Department for Environment, Food and Rural Affairs
DT	Definitive Type
EH	Eviscerator's hand
FDA	Food and Drug Administration
FH	Flayer's hand
FVM	Faculty of Veterinary Medicine
HE	Hektoen enteric Agar
HS	Hand swab
ISO	International Organization for Standardization
LIA	Lysine iron agar
LPS	Lipopolysaccharide
MAFF	Ministry of Agriculture, Fisheries and Food
m.a.s.l.	meters above sea level
MDR	Multiple drug resistant
MLN	Mesenteric lymph node
NCCLS	National committee for clinical and laboratory standards
NMSA	National Meteorological Service Agency

NRL-Salm	National Reference Laboratory for Salmonellosis
OR	Odds ratio
RC	Rumen content
RV	Rapaport-Vassiliadis broth
RVS	Rapaport-Vassiliadis soya broth
SC	Selenite cystine broth
SMID	*Salmonella* detection and identification medium
TSA	Tryptic soy agar
TSI	Triple sugar iron agar
TT	Tetrathionate broth
var	variety
WHO	World Health Organization
XLD	Xylose lysine deoxycholate agar

ACKNOWLEDGEMENS

I would like to express my deepest gratitude to my academic advisors Dr. Bayleyegn Molla and Dr. Ademe Zerihun for their guidance, unreserved moral and material support during the research work, and for taking their time to correct the manuscript.

Alage Agricultural Technical and Vocational Education Collage, Department of Agricultural Extension and Technical Vocational Education of the Ministry of Agriculture and Rural Development and their respective officials deserve special thanks for allowing me to join the graduate program and supported me throughout my study financially and morally.

I would like to express my sincere thanks to members of meat inspection team at the study slaughterhouse for their collaboration during sample collection, the slaughterhouse workers and management especially, Ato Kibret and Ato Tariku for allowing me to work in the slaughterhouse and helping me in arranging the sampling process.

I am deeply grateful to Dr. Kelay Belihu, Associate Dean, for establishing collaborative relationship with AFSSA, which enabled me to complete the research work, and for his moral and administrative support during difficult times. I am very grateful to Dr. Corinne Danan and her colleagues at Agence Française de Securite Sanitaire des Aliments (AFSSA), Unité, Caractérisation et Epidémiologie Bactérienne, Cedex, France for serotyping of our *Salmonella* isolates.

I am also grateful to Prof. A.R.S. Moorthy for his technical support during the laboratory work, Drs. Amene, Gezahegn, Abebayehu and Belay, for their friendship and humor and all staff of the microbiology laboratory, especially Tsehay Tsegaye and Mohammed Hussein for their technical assistance.

Finally, I would like to thank all members of my family for their love and support.

ABSTRACT

A cross-sectional study was conducted on slaughtered cattle at a commercial slaughterhouse in Debre Zeit from October 2005 to February 2006 with the objectives of estimating the prevalence, serotype distribution, the antimicrobial resistance of *Salmonella* isolates from cattle and to investigate the association between some potential sources of *Salmonella* carcasses contamination. A total of 800 samples consisting of (n =100) swabs each, from, hides, flayer' hands, eviscerator's hands, carcasses and holding pens and similar number of samples from caecal and rumen contents and mesenteric lymph nodes, were collected separately. The samples were examined for the presence of *Salmonella* following standard techniques and procedures recommended by the International Organization for Standardization (ISO).

Out of a total of 800 samples, 87 (10.9%) were *Salmonella* positive. Thirty-one (31%) of the hides, 19 (19%) of the rumen contents, 12 (12%) of the holding pens, 8 (8%) of the mesenteric lymph nodes, 7 (7%) of the flayer's hands, 6 (6%) of the caecal contents, 2 (2%) of eviscerator's hands and carcasses each, were positive out of 100 samples examined for each of the sample types. No statistically significant association ($P > 0.05$) could be demonstrated between the bacteriological status of the samples from potential sources of contamination and bacteriological status of the carcasses.

Among the 87 *Salmonella* isolates 85 were identified as serotypes belonging to *Salmonella enterica* subsp *enterica* classified under eight serotypes, while the rest two isolates were grouped under subspecies *salame*. *Salmonella* Anatum (63.2%) and *S*. Newport (20.7%), were the most prevalent. Other serotypes isolated include *S*. Reading (5.7%), *S*. Eastbourne (3.4%), *S*. II 40:b:- (2.3%), *S*. Bredeney, *S*. Typhimurium, *S*. Uganda and *S*. Urbana, 1.1% each. The two predominant serotypes were isolated from, the hide, rumen contents, caecal contents, personnel hands, mesenteric lymph nodes and holding pens. *Salmonella* Urbana and *S*. Eastbourne were the only two isolates recovered from the carcasses.

All of the isolates were tested for susceptibility to a panel of eight antimicrobials. Out of the 87 isolates tested, 36 (41.4%) were resistant. All serotypes, with the exception of *S*. Bredeney, *S*.

Typhimurium and *S*. Urbana, had at least one serotype resistant to one or more of the antimicrobials tested. Multiple antimicrobial resistance was detected for 11.1% of the isolates. Most frequent resistance was encountered for streptomycin (24.1%), followed by tetracycline (20.7%) and amoxicillin/clavulanic acid (2.3%). The most common resistance pattern was a monodrug resistance pattern to streptomycin representing 47.2% of the resistant isolates.

Results of this study showed that *Salmonella* are widespread in cattle at slaughter, personnels' hands and the slaughterhouse environment; however the carcass contamination level was low. The study also indicated the need for further studies to be undertaken to determine risk factors associated with *Salmonella* in cattle and risk factors associated with antimicrobial resistance so that appropriate measures should be taken to reduce *Salmonella* infection and contamination in slaughter animals and thereby minimize the potential foodborne *Salmonella* infection in man.

Key words: Cattle, *Salmonella*, prevalence, antimicrobial resistance, Ethiopia

1. INTRODUCTION

Salmonellosis is considered to be one of the most common foodborne illnesses in humans, with worldwide distribution (CDC, 2000) and is an economically important disease of all animal species. Infection of animals with various serotypes of salmonellae can result in serious clinical disease and always constitutes a vast reservoir for infection of humans. The natural habitat of *Salmonella* is the intestinal tract of humans and other animals. Both water and foods of animal origin have been identified as vehicles for transmission of the organism (Doyle and Cliver, 1990; Radostits *et al*., 1994)

Nontyphoid *Salmonella* serotypes are major causes of foodborne infections worldwide. They seriously affect human health and cause morbidity and mortality. Infections with nontyphoid *Salmonella* serotypes most often result in self-limited acute gastroenteritis that does not require antimicrobial therapy. Nevertheless, approximately 5% of individuals with gastrointestinal illness caused by nontyphoid *Salmonella* serotypes develop bacteraemia (Chiu *et al*., 2004). Children with certain underlying conditions are at increased risk of bacteraemia, which may lead to extra intestinal focal infections. Such conditions include very young age (babies), AIDS, malignancies, immunosuppressive therapy, haemolytic anaemia, and inflammatory bowel disease (Thamlikitkul *et al.*, 1996; Germani *et al.,* 1998). Nontyphoid *Salmonella* bacteraemia is even more serious in adult patients with underlying diseases; these patients are more likely to develop focal infections such as meningitis, septic arthritis, and osteomyelitis; (D'Aoust, 1997; Lai *et al*., 2005).

Nontyphoidal *Salmonella* cause 1.4 million estimated illnesses, 16,430 hospitalisations and 582 deaths each year in the United States (Mead *et al.,* 1999), between 240,000 and 650,000 illnesses in Australia (Sumner *et al*., 2004), 537,000, illnesses in UK (Henson 1997) and 50,000 cases per annum in the Netherlands (van Duijkeren, 2002).

Salmonella infection has been associated with many different food types and the consumption of beef has been associated with a number of outbreaks (Fontaine *et al.,* 1978; Smerdon *et al*, 2001). According to a survey performed by CDC between 1983 and 1987, beef is the second most

common source of human infection (Tauxe, 1991). *Salmonella* has been detected in several locations within dairy farms and slaughterhouses, both before and after sacrifice; the same *Salmonella* clone has been recovered in a dairy herd and in ground meat products following processing (Millemann *et al.,* 2000). It was also shown that the most common serotypes isolated from animal carcasses in the United States were also the most common serotypes found on the corresponding row ground products (Schlosser *et al*., 2000). Therefore, the presence of *Salmonella* in cattle at slaughter and the consequent cross contamination of edible carcass tissue present a significant food safety hazard.

The prevalence of *Salmonella* has been widely reported in slaughtered cattle around the world (Daleel and Frost, 1967; Nazer and Osborne, 1976; Wray and Sojka 1977; Moo *et al.,* 1980; Samuel *et al.,* 1980; Stolle, 1981; Hollinger *et al.,* 1998; McDonough *et al.,* 1999). A number of studies conducted in slaughtered animals, animal products and by-products in Ethiopia indicated the presence of a number *Salmonella* serotypes in the country (Pegram *et al*., 1981; Molomo, 1998; Nyeleti *et al*., 200; Tibaijuka *et al.,* 2003; Alemayehu *et al*., 2003; Woldemariam *et al*., 2005; Molla and Mesfin, 2003; Ejeta *et al*., 2004; Molla *et al.,* 2004; Molla, 2004; Zewdu, 2004; Aragaw, 2005; Demissie, 2005; Molla *et al*., 2006). *Salmonella* was considered as one of the major causes of diarrhoea in humans in different parts of the country (Gedebou and Tassew, 1981; Ashenafi and Gedebou, 1985; Mache *et al.*, 1997; Mache, 2002).

There are over 2500 different *Salmonella* serotypes, and all are considered pathogenic to humans (Wray and Davies, 2003). However, relatively few serotypes are associated with cattle, and of these, *Salmonella enterica* subsp. *enterica* serotype Dublin (*S.* Dublin) and *S. enterica* subsp. *enterica* serotype Typhimurium (*S.* Typhimurium) are the most common in the UK and Ireland (Anononymus, 1996; Egan *et al.,* 1999; MAFF, 2000) and the Netherlands (van Duijkeren, 2002). The presence of *S.* Typhimurium in cattle and the consequent cross contamination of beef carcass tissue is of particular concern as this serotype is one of the most common causes of *Salmonella* infection in developed countries (Gomez *et al.,* 1997).

In addition to causing infection, many *S.* Typhimurium isolates (although not exclusively isolates of this serotype) have developed resistance to multiple antibiotics. Of particular note is *S.*

Typhimurium definitive type (DT) 104. Many isolates of this phage type are resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (ACSSuT), with an increasing number of isolates showing resistance to trimethoprim and fluoroquinolones (Threlfall *et al.*, 1997; Piddock, 2002).

Given the association of certain bovine *Salmonella* serotypes with food poisoning and the likelihood that some isolates may be resistant to multiple antimicrobials, a complete understanding of the risk posed by this pathogen during beef processing requires that the serotype and antibiotic resistance profile of isolates be determined in addition to the prevalence (McEvoy *et al.*, 2003).

Despite the presence of a number of published information on different food items, little information is available on the status of *Salmonella* in apparently healthy slaughtered cattle in commercial abattoirs in Ethiopia. Therefore this study was undertaken on slaughtered cattle and slaughter environment with the following objectives:

- Estimate the prevalence of *Salmonella*
- Establish serotype diversity in slaughtered cattle
- Determine the antimicrobial resistance patterns of *Salmonella* isolates and
- Find out some of the sources of carcass contamination for future control of such contaminations

2. LITRATURE REVIEW

Salmonella are Gram-negative, nonsporeforming, facultatively anaerobic rods belonging to the family *Enterobacteriaceae* (Jay, 2000; Patterson and Isaacson, 2003). Although members of this genus are motile by peritrichous flagella, non-flagellated variants, such *S.* Pullorum and *S.* Gallinarum, and non-motile strains resulting from dysfunctional flagella do occur. Salmonellae are chemoorganotrophic, with an ability to metabolise nutrients by the respiratory and fermentative pathways (D'Aoust, 1997).

The genus *Salmonella* consists of resilient microorganisms that readily adapt to extreme environmental conditions. Salmonellae actively grow within a wide temperature range between 8^{o}C and 45^{o}C and pH range of 4 to 8, with an optimum temperature of 37^{o}C and pH of 6.5 to 7.5. They do not survive at temperatures higher than 70^{o}C. They catabolize D-glucose and other carbohydrates (D'Aoust, 1997; Acha and Szyfres, 2001).

2.1. Taxonomy and nomenclature

According to the scheme proposed by Le Minor and Poppof in 1987, the genus *Salmonella* consists of two species; (1) *S. enterica* which is divided into six sub species: *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica subsp*. *houtenae* (IV) and *S. enterica* subsp. *indica* (VI); and (2) *S. bongori* (formerly called *S. enterica* subsp. *bongori* (V) (Popoff and Le Minor, 1997). The Judicial Commission of the International Committee on Systematics of Prokaryotes accepted the proposed recommendations after years of debate on the status to be given to *S.* Typhi (Brenner *et al*., 2000; Judicial Commission, 2005).

Historically, species names were arbitrarily given to serotypes for convenient reasons in medical practice. Some serotype names denote syndrome (*S. typhi*) or relationships (*S.* paratyphi A, B, C). Other names were correlated with syndrome and host specificity, which was right in some cases

(*S. abortusovis, S. abortusequ*i) or wrong in other cases (*S. typhimurium, S. choleraesuis*). To avoid sources of possible confusion, names indicating geographical origin of the first strain of the new serotype (S. *london, S. panama S.* kentucky) were then used (Popoff and Le Minor, 1997). For named serotypes, to emphasize that they are not separate species, the serotype name is not italicized and the first letter is capitalized. At the first citation of a serotype the genus name is given followed by the word "serotype" or the abbreviation "ser." and then the serotype name (for example, *Salmonella* serotype or ser. Typhimurium). Subsequently, the name may be written with the genus followed directly by the serotype name (for example, *Salmonella* Typhimurium or *S.* Typhimurium) (Popoff and Le Minor, 1997: Brenner *et al*., 2000).

The antigenic formulae of *Salmonella* serotypes are defined and maintained by the World Health Organization (WHO) Collaborating Center for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France, and new serotypes are listed in an annual updates of the Kauffmann -White scheme. Before 1966 all serotypes in all subspecies except subspecies IIIa and IIIb were given names. In 1966 the WHO Collaborating Center began naming serotypes only in subspecies I and dropped all existing serotype names in subspecies II, IV, and VI and *S. bongori* from the Kauffmann-White scheme (Popoff and Le Minor, 1997).

2.2. Detection of *Salmonella*

The detection of *Salmonella,* from products intended for human consumption or feeding of animals, necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in selective liquid media, plating out and recognition and confirmation (ISO 6579, 1998).

2.2.1. Pre-enrichment in non-selective liquid medium

Means of enrichment for *Salmonella* can have profound effects on the ability to recover them from animal sources, foods, water or environmental samples. The method selected will vary with regard to the need for non-selective nutrient broths such as buffered peptone water (BPW) or trypticase soy broth for resuscitating damaged cells from heat-stressed, desiccated, or otherwise less than ideal samples (Maddox, 2003).

The test sample is initially inoculated into a non-inhibitory liquid medium to favour the repair and growth of stressed or sub lethally-injured salmonellae arising from exposure to heat, freezing, desiccation, preservatives, high osmotic pressure or wide temperature fluctuations (Andrews, 1989; D'Aoust, 1989). This step in the detection of *Salmonella* involves inoculation of buffered peptone water (ISO 6579, 1998) or lactose broth (FDA, 1998) with the test portion, and incubation at 35^{o}C or 37^{o}C for 16 h to 20 h (ISO 6579, 1998) or 24 ± 2 h at 35°C (FDA, 1998).

2.2.2. Enrichment in selective liquid media

Enrichment in selective liquid medium may follow an initial enrichment in non-selective liquid media or it may be used as an initial step in specimens containing high numbers of competing bacteria such as faeces or ground meats to prevent overgrowth by coliforms that can readily out-compete the *Salmonella* (Maddox, 2003).

Enrichment is done by the transfer of 0.1 ml of the culture obtained in nonselective pre-enrichment medium to a tube containing 10 ml of a Rappaport-Vassiliadis magnesium chloride malachite green medium (RV medium) (ISO 6579, 1998; FDA, 1998) and transfer of another 10 ml to a flask containing 100 ml of selenite cystine medium (ISO 6579, 1998) or 1 ml mixture to 10 ml tetrathionate (TT) broth (FDA, 1998). The inoculated RV medium is incubated at 42^{o}C for 18 to 24 h (ISO 6579, 1998) or 24 ± 2 h at 42 ± 0.2°C (FDA, 1998), and selenite cystine medium at 35^{o}C to 37^{0}C 18h to 24h (ISO 6579, 1998). The TT broth is incubated at 24 ± 2 h at 43 ± 0.2°C (FDA, 1998).

2.2.3. Plating out and identification

There is a range of selective differential agars, which distinguish enteric bacteria and have been applied to the detection of salmonellae. These include xylose-lysine-deoxycholate agar (XLD), brilliant green agar, modified brilliant green agar, Hektoen- enteric agar, mannitol-lysine-crystal violet-brilliant green agar, *Salmonella-Shigella* agar, deoxycholate-citrate agar, and bismuth sulphite agar (Anonymous, 1990; Perry *et al*., 1999). The selectivity of these agars is due to the presence of bile salts (or other surfactive compounds) and inhibitors such as brilliant green. Differentiation of most salmonellae from other organisms, particularly members of the family *Enterobacteriaceae*, relies on the ability to produce hydrogen sulphide and/or the inability to ferment lactose and thus lower the medium pH (Cooke *et al*., 1999).

Salmonella agars incorporating chromogenic substrates are now commercially available. Rambach agar (Rambach, 1990) incorporates propylene glycol, which is metabolised by *Salmonella* spp. to give acid products detected by neutral red, together with a chromogenic β-galactosidase substrate (5-bromo- 4-chloro-3-indolyl-β-D-galactopyranoside [X-Gal]). This is hydrolysed by many non-*Salmonella* species to give a blue, insoluble product. *Salmonella* detection and identification (SMID) agar (Cooke *et al*., 1999) also uses a chromogenic β-galactosidase substrate together with a glucuronate, which is metabolised by salmonellae. Another medium called αβ- chromogenic medium (ABC medium), which contains two substrates, 3,4-cyclohexenoesculetin-β-D-galactoside and 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside, to facilitate the selective isolation of *Salmonella* spp. has been described (Perry *et al*., 1999).

Many commercially available methods to enhance detection of *Salmonella* organisms by means of antibody capture have been increasingly utilized to improve the sensitivity and specificity of antigens detection. Immunomagnetic beads, coated with anti-*Salmonella* LPS or flagella antibodies, have been used to capture *Salmonella* from enrichment broth suspensions. The beads are then plated by rolling them across the agar surface of differential or selective media (or both) such as XLD or BG (Maddox, 2003).

Survey results published by Waltman and Mallinson (1995) indicated great disparity in methods among laboratories performing *Salmonella* culture; none of the seventy-four respondent laboratories reported using the same methods for culture and isolation. The report also indicated fourteen different media and various incubation which, greatly affect the sensitivity of detection.

In standard protocols, such as the ISO procedures, plating out and identification involves inoculation of two selective solid media (ISO 6579, 1998);

- Brilliant green/phenol red agar, unless the International Standard appropriate to the product to be examined, or other specific considerations (for example the isolation of lactose-positive *Salmonella*), require substitution of some other medium as the one for obligatory use;

- The choice of the second medium is left to the discretion of the testing laboratory, unless there is a specific international standard, relating to the product to be examined, which specifies the composition of this second medium.

In the recent ISO protocol, ISO 6579 (2002) Muller-Kauffmann tetrathionate novobiocin (MKTTn) broth is used as a replacement for selenite cystine broth for selective enrichment in the previous protocol. The ox bile and brilliant green within MKTTn broth improves selectivity, while the addition of novobiocin improves the inhibition of *Proteus spp.* In this protocol XLD is used as a replacement for BPLS in ISO 6579 (1998), while the choice of the second plating out medium is left for the investigator.

According to FDA (1998), 3 mm loopful (10 µl) incubated TT broth and RV medium is streaked on bismuth sulfite (BS) agar, xylose lysine deoxycholate (XLD) agar, and Hektoen enteric (HE) agar. The host-adapted serotypes from swine and poultry are more fastidious than most of the other commonly isolated serotypes so they do not tolerate brilliant green agar; although most strains of *S.* Choleraesuis will grow on modified brilliant green agar (Quinn *et al*., 1999).

2.2.4. Confirmation

Presumptive *Salmonella* colonies are subcultured and confirmed by means of appropriate biochemical and serological tests. For confirmation typical or suspect colonies are streaked onto the surface of pre-dried tryptic soy agar plates, in a manner, which will allow well-isolated colonies to develop. Pure cultures are used for biochemical and serological confirmation (ISO 6579, 1998).

According to FDA (1998), lightly touch the very centre of presumptive colony to be picked with sterile inoculating needle and inoculate triple sugar iron agar (TSI) slant by streaking slant and stabbing butt. Without flaming, inoculate lysine iron agar (LIA) slant by stabbing butt twice and then streaking slant. Streak TSI agar cultures that appear to be mixed on MacConkey agar, HE agar, or XLD agar in order to purify.

2.2.4.1. Biochemical confirmation

Salmonellae 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 the sole carbon source, generally produce hydrogen sulphide, decarboxylate lysine and ornothine, and do not hydrolyze urea (D'Aoust, 1997; Quinn *et al*., 1999).

The majority of salmonellae are non-lactose fermenters and produce pale colonies on MacConkey agar and an alkaline reaction in the medium. However, it must be remembered that some strains of *S. arizonae* are lactose positive and strains of *S.* Typhimurium have been encountered carrying plasmids with genes coding for lactose fermentation. Most salmonellae give an alkaline reaction in brilliant green agar and have red colonies. On XLD medium the majority of *Salmonella* serotypes produce hydrogen sulphide and have red colonies with a black (H_2S) centre. Colonies characteristic for *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* in TSI agar is a red (alkaline) slant, yellow (acid) butt and superimposed

(black) H_2S production (R/Y/ H_2S^+). The test for lysine decarboxylation is positive. However, *S.* Choleraesuis does not produce H_2S although *S.* Choleraesuis biotype Kunzendorf is H_2S positive. If the reaction in TSI and lysine decarboxylase broth is equivocal, further biochemical tests should be carried out or an identification system such as API 20E should be used (Analytab products) (D'Aoust, 1997; Quinn *et al*., 1999). *Salmonella* generally are β-galactosidase, Voges-Proskauer and indole negative (ISO 6579, 1998).

The dynamics of genetic variability arising from bacterial mutations and conjugative intra and intergeneric exchange of plasmids encoding determinant biochemical traits continue to reduce the proportion of typical *Salmonella* biotypes. This is shown by the presence of biotypes capable of fermenting lactose and sucrose, that possess urea activity, that produce indole and that readily grow in the presence of potassium cyanide. Clearly, the prevalence of *Salmonella* species as a biochemically homogeneous group of microorganisms is rapidly diminishing. The situation will likely lead to a reassessment of the diagnostic value of biochemical traits and to their likely replacement with molecular technologies targeted at the identification of stable genetic loci and/or their products that are unique to the genus *Salmonell*a (D'Aoust, 1997).

2.2.4.2. Serological confirmation

The antigenic classification or serotyping of *Salmonella* used today is a result of extensive studies of antibody interactions with bacterial surface antigens by Kauffmann and White (Popoff and Le Minor, 1997). Three kinds of surface antigens, somatic O antigens, flagellar H antigens, and capsular Vi, determine the reactions of the organisms to specific antisera (Popoff and Le Minor, 1997). Although extensive serotyping of all surface antigens can be used for formal identification, most clinical microbiological laboratories perform a few simple agglutination reactions to define specific O antigens into serogroups, designated groups such as A, B, C1, C2, D, and E (Popoff and Le Minor, 1997).

2.2.5. Typing of *Salmonella*

2.2.5.1. Serotyping

Serotyping is based on the O (somatic) and H (flagellar) antigens and a slide agglutination test is used. Rare strains of *S.* Dublin have a Vi (virulent) capsular antigen that can mask the cell wall (O) antigens. Boiling a suspension of *S.* Dublin for 10-20 minutes will destroy the Vi antigen. 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 rough strains and will invalidate the serotyping (Quinn *et a*l., 1999).

Smooth *Salmonella* to be serotyped is emulsified in a drop of 0.85 % saline on a clean microscope slide. A drop of antiserum is added to, and mixed well with the *Salmonella* suspension. The slide is rocked gently for about 30 seconds and the antigen-antibody mixture examined for agglutination. The *Salmonella* is first tested against antisera to the O (somatic antigens) and then the H (flagella) antigens (Quinn *et a*l., 1999). Flagellar antigens are of two types: specific phase or phase 1, and group phase or phase 2. Phase 1 antigens are shared with only a few other species or varieties of *Salmonell*a; phase 2 may be more widely distributed among several species (Jay, 2000).

Some serotypes have several different phenotypes, and their identification can be important in epidemiologic investigation. Phage typing is also useful for some serotypes (Acha and Szyfres, 2001). Approximately 2,500 different *Salmonella* serotypes have been described, and the number increases annually as new serotypes are recognized (Wray and Davies, 2003).

2.2.5.2. Phage typing

Phage typing schemes for *Salmonella enterica* serotypes are based on patterns of lysis produced by distinct phages isolated from a variety of sources (Olsen *et a*l., 1993; Quinn *et a*l., 1999; Heuzenroeder *et al.,* 2004). Pure cultures of bacteria are flooded onto plates and suspensions of typing phages are spotted onto the plates. Strains that are susceptible to infection by the same phages are allocated to the same phagetype. As this typing method is cheap and labour

inexpensive, it is normally the second method to be applied in the study of *Salmonella* epidemiology, and phage typing schemes have been developed for many important *Salmonella* serotypes. Phage typing is the principal method of typing in *S.* Enteritidis and *S.* Typhimurium (Olsen *et a*l., 1993). Phage typing has been used to subdivide isolates within serotypes Typhi, Typhimurium, Enteritidis, Virchow, Hadar and Heidelberg. Although phage typing is essential for the subdivision of *Salmonella* serotypes, the method can prove inadequate for serotypes in which a small number of phage types predominate (Heuzenroeder *et al.,* 2004).

2.3. Epidemiology

The epidemiology of salmonellosis as a disease of animals and zoonosis is complex. The epidemiological patterns differ greatly between geographical areas depending on climate, population density, land use, farming practices, food harvesting and processing technologies, and consumer habits (Radostits *et al*., 1994).

Managemental, physiological and nutritional factors, as well as intercurrent infections, not only predispose animals to salmonellosis, but they may also precipitate clinical disease in infected cattle and the excretion of the bacteria by carrier animals. Predisposing factors include unhygienic conditions, overcrowding, transportation, insufficient intake of colostrums, inadequate availability of water and food, inclement weather, hospitalisation, surgery and parturition. Intercurrent infections that may play a role include bovine virus diarrhoea and mucosal disease, infectious bovine rhinotracheitis and *E.coli,* or infestation with parasites such as *Fasciola hepatica.* The effects of these ‘stress factors’ are probably related to the suppression of cell-mediated immunity (Venter *et al.,* 1994).

2.3.1. Distribution

Although primarily intestinal bacteria, *Salmonella* are widespread in the environment and commonly found in farm effluents, human sewage, and in any material subject to faecal contamination (Wray and Davies, 2003). Salmonellosis has been recognized in almost all

countries but appears to be most prevalent in areas of intensive animal husbandry. Although the disease can affect all species of domestic animals, young and pregnant animals are the most susceptible. Many animals may also be infected without showing signs of illness (Radostits *et al*., 1994).

Although a large number of *Salmonella* serotypes exist, the overall increase in the number of infections is of relatively few emerging serotypes and phage types. Over periods of several years, certain *Salmonella* types have risen and (sometimes) fallen within large geographic regions (Cogan and Humphrey, 2003). These meta-outbreaks are facilitated through the acquisition, by specific types, of new traits that make them well adapted to spread, as well as through changes to human society, seen for instance with modern intensified farming and food production methods and global trade with live breeder animals (Tauxe, 1999; Swartz, 2002). Two prominent examples are the international spread of *S.* Enteritidis infections through chicken eggs (Rodrigues, 1990; Tauxe, 1999; Cogan and Humphrey, 2003) and the emergence over the last 2 decades of multidrug resistant *Salmonella* Typhimurium DT104 (Helms *et al*., 2005). It has been postulated that the virtual eradication of *S.* Gallinarum from the UK and US through testing and culling of sero-positive hens, followed by vaccination, left a niche that was filled by *S.* Enteritidis (Cogan and Humphrey, 2003).

Salmonella Typhimurium and *S.* Dublin appears to be the commonest serotype isolated from cattle, although the distribution of these serotypes may differ between countries and over a period of time (Wray and Davies, 2000). Infection of cattle with *S.* Dublin is more frequent than with *S.* Typhimurium in Denmark and Sweden, while the converse is the case in France (Wray and Davies, 2000). In the Netherlands, *S.* Typhimurium was the most common bovine isolate prior to 1991. Since 1991, however, serotype Dublin has been the most common serotype (Wray and Davies, 2000; Van Duijkeren *et al*., 2002). The most common serotype reported from cattle during the period 1999-2000 in the United States was *S.* Typhimurium followed by serotypes *S.* Anatum and *S.* Dublin (Wray and Davies, 2003), while a report during the same period in the UK, showed *S.* Dublin followed by *S.* Typhimurium (MAFF, 2000).

2.3.2. Host range

For epidemiological purposes, the salmonellae can be placed into three groups based on their host adaptation (Jay, 2000):

- Those that infect humans only: These include *S.* Typhi, *S.* Paratyphi A and *S.* Paratyphi C.

- The host adapted serotypes (some of which are human pathogens and may be contracted from foods): included are *S.* Gallinarum (poultry), *S.* Dublin (cattle), *S.* Abortusovis (sheep), and *S.* Choleraesuis (swine)

- Unadapted serotypes (no host preference). These are pathogenic for humans and other animals, and they include most foodborne serotypes.

Although *S. enterica* subspecies I contains more than 1,400 different serotypes (D'Aoust, 1997), only one or a few are associated with the majority of cases of illness in a particular avian or mammalian species (Table 1). These serotypes show different degrees of host adaptation. Pathogens that lack host specificity, such as *S.* Typhimurium and *S.* Enteritidis, tend to be more frequently associated with disease in young animals than in adults, suggesting that they are not optimally adapted to cope with a fully mature immune system. Serotypes that are host specific, on the other hand, have acquired the ability to breach defense mechanisms in full-grown animals, as shown by their association, at similar rates, with illness in all age groups (Table 1). Furthermore, host-specific serotypes tend to be more virulent, as illustrated by the fact that they cause higher mortality rates (Bäumler *et al*., 1998).

Table 1. Diseases caused by *Salmonella* subspecies I serotypes in humans and higher vertebrates (Bäumler *et al*., 1998)

Host species	Disease	*S. Enterica* subspecies I serotype(s) most frequently encountered	Most susceptible age groups	Typical symptoms or sign(s) of disease
Humans	*Salmonella* enteritis	Typhimurium, Enteritidis	Children (< 4 yr)	Diarrhoea, dysentery, fever
	Typhoid fever	Typhi[c]	Children and adults	Septicaemia, fever[a]
	Paratyphoid fever	Sendai; Paratyphi A, B, and C[c]	Children and adults	Septicaemia, fever[a]
Cattle	Salmonellosis	Typhimurium	Calves (< 8 wk)	Diarrhoea, dysentery, septicaemia, fever
		Dublin	Calves and adult cattle	Diarrhoea, dysentery, septicaemia, abortion, fever
Poultry	Pullorum disease	Pullorum[c, d]	Newly hatched birds	Diarrhoea, septicaemia
	Fowl typhoid	Gallinarum[c, d]	Growing stock and adults	Diarrhoea, comb discoloration, septicaemia
	Avian paratyphoid	Enteritidis, Typhimurium	Newly hatched birds	Diarrhoea, septicaemia
Sheep	Salmonellosis	Abortusovis[c]	Adult sheep	Septicaemia, abortion, vaginal discharge
			Lambs	Diarrhoea, dysentery, septicaemia
		Typhimurium	Lambs	Diarrhoea, dysentery, septicaemia
Pigs	Pig paratyphoid	Choleraesuis[c]	Weaned and adult pigs	Skin discoloration, septicaemia, fever[b]
	Salmonellosis	Typhimurium	Weaned pigs (< 4 mo)	Diarrhoea
	Chronic paratyphoid	Typhisuis		Intermittent diarrhoea
Horses	Salmonellosis	Abortusequi[c]	Adult horses	Septicaemia, abortion
			Foals	Diarrhoea, septicaemia
		Typhimurium	Foals	Diarrhoea, septicaemia
Wild rodents	Murine typhoid	Typhimurium, Enteritidis		Septicaemia, fever

a Diarrhoea develops only in about one third of typhoid fever patients and usually several days after the onset of fever.

b Diarrhoea is not a typical sign of pig paratyphoid but may develop by the third or fourth day of disease.

c These serotypes have been most frequently associated with illness in the preantibiotic era but are now rare or have been eradicated in most developed countries.

d Gallinarum and Pullorum are considered biotypes that belong to the same serotype.

2.3.3. Sources of infection and transmission

2.3.3.1. Route of infection

Although experimental aerosol transmission has been produced in esophgotomized calves and piglets by researchers (De Jong and Ekdahl, 1965; Fedorka-Cray *et al.,* 1995), the usual route of infection is through feco-oral route of exposure. A number of experiments have shown that oral doses ranging from 10^6 to 10^{11} *S.* Dublin and 10^4 to 10^{11} *S.* Typhimurium are necessary to cause disease in healthy cattle. These doses are likely to be higher than those encountered under natural conditions, where concurrent diseases and stress contributes (Wray and Sojka, 1977).

2.3.3.2. Sources of infection for animals

The most important sources of *Salmonella* organisms are clinically affected and carrier animals, and faecally contaminated environments, water and feedstuffs (Glickman *et al*., 1981). Clinically affected animals excrete large numbers of bacteria, particularly in their faeces. Affected animals may excrete 10^8 to 10^{10} salmonallae per gram of faeces. Considering that cattle produce about 20 to 28 kg faeces a day, clinically affected cow may shed 10^{14} salmonellae each day thereby exponentially amplifying environmental contamination (Glickman *et al*., 1981). These animals may later become carriers and continue to excrete the organism in their faeces, contaminating the pasture and the environment for long period of time. Apart from faecal excretion of salmonellae, organisms may also be excreted in urine and saliva of diseased calves. *Salmonella* Dublin may be excreted in the milk, faeces, and urine of cows for up to four weeks after parturition or abortion (Venter *et al*., 1994).

Most infections are introduced into *Salmonella*-free herds by the purchase of infected cattle, either as calves for intensive rearing or adult cattle for replacements. Purchased animals may have acquired infection on their home-farm premises, on transit or on dealers' premises (Wray, 1991).

Salmonella can be isolated regularly from animal feeds and their ingredients, which include both animal and vegetable proteins (Krytenburg, 1998). Therefore, feed is a major potential route by which new infections may be introduced into a herd or flock (Davies and Hinton, 2000). Many new serotypes have been introduced into countries in imported feed ingredients (Clark *et al.*, 1973). Basic cereal ingredients such as barley or maize are not considered to be particularly susceptible to *Salmonella* contamination but may be contaminated, during storage, by wild animals, especially rodents or birds. Contaminated hay may also serve as a source of infection (Glickman *et al*., 1981).

Salmonella are widespread in the environment and can exist in many niches. As a consequence, *Salmonella* perhaps should be thought of as an environmental organism whose dissemination is likely to continue and increase in the future (Murray, 1991). Farm buildings may become directly contaminated with *Salmonella* following outbreaks of disease or colonization of animals, or indirectly contaminated from other sources such as contaminated water used for cleaning or from wild animals and birds. Persistent contamination of houses and transport vehicles is an important factor in the maintenance and spread of *Salmonella* in animal populations, and the organism may persist in dry livestock buildings for up to 30 weeks (Davis and Wray, 2000).

Salmonella may survive for long periods in contaminated faeces and slurries where their survival is dependent on a number of factors, especially climatic conditions. In moist, uncomposted faces, *Salmonella* may survive for 3-4 months in temperate climates and for longer periods in hotter climates. However, in properly composted faeces the high temperature produced rapidly kills *Salmonella* (Wray and Davies, 2000).

Numerous publications have recorded the isolation of *Salmonella* from a wide range of animals, birds and arthropods. *Salmonella* infections have been detected in many species of wild birds (Johnson *et al.,* 1979; Tizzard *et al.,* 1979). Seagulls have been found to spread *Salmonella* on to pastures where domestic animals may acquire infection (Johnson *et al.,* 1979; Tizzard *et al.,* 1979). Similarly, flies and other insects have been shown to be vectors of *Salmonella* (Kopanic *et al.,* 1994). Evans and Davies (1996) found that wild birds and cats were possible vectors of *S.* Typhimurium DT104, particularly if they have access to feed stores. Rodents have been

implicated in outbreaks in a dairy herd (Tablante and Lane, 1989) and in a beef herd (Hunter *et al.*, 1976).

2.3.3.3. Sources of infection for human beings

Salmonella enterica serotypes causing typhoid fever do not have an animal reservoir and persist in the human population by person-to-person transmission (Rabsch, 2003). In contrast nontyphoid *Salmonella* serotypes causing gastroenteritis in humans are most often transmitted through the food chain by contamination of poultry and eggs, pork, beef and dairy products and increasingly in the United States by vegetables and fruits that are irrigated with *Salmonella* contaminated water or cross contamination through the use of dirty utensils, in both commercial processing plants and household kitchens (Mead *et al*., 1999). Meat can become contaminated in abattoirs by means of contaminated equipment and utensils during skinning and butchering. Contaminated public or private water supplies are important sources of infection in typhoid fever (*S*. Typhi) and, less frequently, in other *Salmonella* infections (Acha and Szyfres, 2001). Transmission of *Salmonella* spp. to human beings by direct or indirect contact to animals has been reported (Fey *et al*., 2000; Hendriksen, 2004; Wright *et al*., 2005).

3.3.4. Carrier state

Adult cattle are important in the dissemination of *Salmonella* as they act as carriers, particularly of *S*. Dublin (Wray, 1991). Carrier animals harbour organisms in their mesenteric lymph nodes, gut associated lymphoid tissue, macrophages in the lamina propria of the intestine, and the gall bladder. Stressed animals shed the organisms more readily and in higher number than non-stressed animals (Venter *et al*., 1994).

Cattle infected with *Salmonella* Dublin may eventually lose the infection or remain life long carriers, shedding the organism intermittently or continuously in large number in their faeces (Richardson, 1975). It may also become a latent carrier with infection persisting in lymph nodes or tonsils but no salmonellae in the faeces. The importance of the latent carriers is that they can become active carriers or even clinical cases under stress, especially at calving time (Radostits *et*

al., 1994). In contrast cattle carrying *S*. Typhimurium shed the bacteria for a limited period only, in most instances no longer than three or four months (Richardson, 1975). It was shown that animals might even become passive carriers, which constantly pick up infections from the pasture or the calf pen floor, but are not invaded so that when they are removed from the environment the infection disappears. These animals probably multiply the salmonellae without becoming permanent carriers (Radostits, 1994).

Although the carrier status in Ethiopian cattle population has not been conducted nationwide, cross-sectional surveys conducted on animals presented to different abattoirs for slaughter, indicated that salmonellae are isolated from faeces and mesenteric lymph nodes of slaughtered animals. Pegram *et al*. (1981) isolated *Salmonella* from 2.1% of mesenteric lymph nodes of adult cattle presented for slaughter at Addis Ababa abattoir. More recently, a study conducted at Addis Ababa abattoir revealed that 2.1% of the cattle shed the organisms in their faeces while 3.8% of the animals harbour the organism in their mesenteric lymph nodes (Nyeleti *et al*., 2000). Alemayehu *et al*. (2003) isolated *Salmonella* from 0.6 and 0.9% of faeces and mesenteric lymph node respectively, from animals presented for slaughterer at the FVM's abattoir.

2.4. Clinical picture

2.4.1. In cattle

In cattle the enteric and septicemic syndromes of salmonellosis are more common than the abortion syndrome. One or more of the syndromes may occur simultaneously in an outbreak of salmonellosis in a herd, or even in a single affected animal. *Salmonella* Dublin is more often the cause of septicaemia than *S*. Typhimurium. And adult animals are less likely than calves to suffer from septicemic infections. Calves may develop peracute, acute or chronic salmonellosis. The peracute disease is usually a septicaemic condition. It is often fatal, and calves may die suddenly without being seen to be ill. Some however develop enteritis and diarrhoea in addition to septicaemia. When the course of septicaemia is protracted, signs of hepatitis, pneumonia,

meningoencephalitis, polyarthritis and osteomyelitis may develop (Venter *et al*., 1994, Wray and Davies, 2000).

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, inappetence, lethargy, diarrhoea, polypnoea, and frequently a serous nasal discharge followed by a slight cough. Scouring may only occur terminally. The faeces of affected calves are foul smelling, have a putty-like consistency, and contained large amounts of mucus, sloughed mucosal and flecks of blood. At this time the body temperature may be normal or subnormal. The mortality rates are 5 to 10 per cent and may reach as high as 75 per cent 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. Diarrhoea is not always present and sign of chronic pneumonia with persistent coughing may occur (Wray and Davies, 2000). Calves that survive either peracute, acute or chronic disease may develop pneumonia, meningoencephalitis, purulent polyarthritis, and osteomyelitis of the vertebrae and bones of the distal parts of the limb, resulting in lameness, paresis or even almost complete paraplegia. Dry gangrene of the skin of the lower limbs and the tips of ears and tail, resembling ergotism, is rarely encountered (Venter *et al*., 1994)

Adult cattle generally contract either acute or subacute enteric salmonellosis, and pregnant animals may abort (Venter *et al*., 1994). During the early stages of the acute enteric disease, severely affected animals show fever, depression, inappetence, and a drop in milk yield. These signs are followed by diarrhoea which is foul-smelling, the faeces being mucoid and usually containing clots of blood and shreds of necrotic intestinal mucosa. Signs of colic, congested mucus membranes and dehydration may be evident. Most of these signs are associated with endotoxemia induced by 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 is 50 per cent, but may be higher. Complete recovery may take up to two months. Similar but less severe signs are present in animals suffering from subacute

enteric salmonellosis, and most affected animals recover without treatment. (Radostits *et al*., 1994; Venter *et al*., 1994)

Salmonella Dublin in particular, but also other serotypes, may cause abortion in cows 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, septicaemia and/or placentitis being the cause of death of the foetus. Retention of the placenta occurs in approximately 70 per cent of cows that abort, but subsequent fertility is not affected (Venter *et al*., 1994).

2.4.2. In humans

Salmonella infections can manifest in five different clinical forms; enteric fever, gastroenteritis, bacteraemia, extra intestinal localized infection and in a chronic enteric or urinary carrier state. The major risk factors for nontyphoidal salmonellosis and bacteraemia are extremes of age, alteration of the endogenous bowel flora of the intestine, diabetes mellitus, malignancy, autoimmune disorders, reticuloendothelial blockade, HIV infection and therapeutic immunodeficiency (Brown and Eykyn, 2000).

Enteric (typhoid) fever is a serious human disease associated with the typhoid and paratyphoid strains which are particularly well adapted for invasion and survival within human host. Infection with nontyphoid salmonellae commonly results in gastroenteritis, which appears 6 to 72 hours after contact with the invasive pathogen (Urfer *et al*., 2000). The clinical condition is generally self-limiting, and remission of the characteristic nonbloody diarrhoeal stools and abdominal pain usually occurs within 5 days of onset of symptoms. Human infections with nontyphoid strains can also degenerate into systemic infections and precipitate various chronic conditions such as aseptic reactive arthritis, and ankylosing spondylitis. Preexisting physiological, anatomical and immunological disorders in human hosts can also favour severe and protracted illness through the inability of host defence mechanisms to respond effectively to the presence of invasive salmonellae (D'Aoust, 1997; Lai *et al*., 2005)

2.5. Treatment and control

2.5.1. Treatment

In severe cases where inflammation of the bowl mucosa, results in dysentery, and the passage of mucosal casts, fluid, electrolyte, and protein loss may progress rapidly and become life threatening. In such cases, replacement of fluid therapy is the cornerstone of treating salmonellosis in cattle. Additional therapies include non-steroidal anti-inflammatory drugs to block the effect of endotoxin and antibiotics to treat the associated bacteraemia (Radostits *et al*., 1994).

There are differences of opinion among veterinarians about the rationale and wisdom of treating cases of salmonellosis with antimicrobials because of their efficacy and the likelihood of producing carriers. The general view of most practitioners is that prompt treatment of clinical cases with broad-spectrum antibiotics is beneficial. Aggressive treatment early in the course of the disease, especially in calves, where infection may become septicaemic, is required (Wray and Davies, 2000). For best response to treatment, antimicrobial therapy should be based on the antimicrobial sensitivity of salmonellae isolated from the tissues of a necropsied herdmate (Radostits *et al*., 1994; Venter *et al*., 1994; Wray and Davies, 2000). Oral administration of antimicrobial drugs in cattle is a satisfactory method of treatment, but it must be used with caution. Some antimicrobial drugs such as neomycin, chloramphenicol, tetracycline and ampicillin may cause malabsorpton diarrhoea after three days of treatment. Disturbances of the gastrointestinal flora by the use of antimicrobial drugs in the presence of resistant *Salmonella*, may involve the intestinal flora responsible for restricting the multiplication of *Salmonella*, resulting in overgrowth and conversion of an enteric infection into a septicaemic form (Radostits *et al*., 1994; Venter *et al*., 1994).

In humans, the clinical condition caused by nontyphoid salmonellae is generally self-limiting. The successful treatment of uncomplicated cases of enterocolitis may require only supportive therapy such as fluid and electrolyte replacement. The use of antimicrobials in such episodes is contraindicated because it tends to prolong the carrier state and the intermittent excretion of

salmonellae. Moreover, digestive symptoms after clearance of the infectious agent are significantly higher in patients treated with antimicrobials during acute gastroenteritis (D'Aoust, 1997). However, antimicrobial therapy is indicated to treat invasive *Salmonella* infections and in patients with certain underlying conditions such as extremes of age, immunosuppressive diseases, and patients undergoing immunosuppressive therapy, which may lead to extraintestinal focal infections (Lai *et al*., 2005). Antimicrobial therapy is also necessary for the treatment of *S.* Choleraesuis infections in countries such as Taiwan, where approximately 78% of the infected individuals developed bacteraemia with little or no intestinal involvement (Chang *et al*., 2005).

2.5.2. Control and prophylaxis

Control measures could be directed against the host the agent or the environment. Measures directed towards the host include, increasing the resistance of animals in high-risk groups by immunization and appropriate feeding to maintain intestinal flora in a balanced state. Separation of age groups and the 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 (Radostits *et al*., 1994; Venter *et al*., 1994).

The use of vaccine in the control of salmonellosis has been considered to be valuable only as an auxiliary provided that *Salmonella* population be restrained to reasonable levels (Radostits *et a*l., 1994). Both live attenuated vaccines produced from rough strains and bacterins precipitated on aluminum hydroxide are commercially available. Comparative vaccine trials indicated that modified live *Salmonella* vaccines provide greater protection against virulent *Salmonella* than do *Salmonella* bacterins (Radostits *et a*l., 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, equipment and transport vehicles should also be cleaned and disinfected (Venter *et al.*, 1994).

Measures aimed at the environment include, institution and maintenance of hygiene and good animal husbandry practices such as regular removal of faeces, 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 the prevention of overcrowding (Venter *et al*., 1994).

2.6. Antimicrobial resistance of *Salmonella*

The introduction of antimicrobial agents in human clinical medicine and animal husbandry has been one of the most significant achievements of the 20th century. The first antimicrobial agents were introduced in the 1930s, and a large number of new compounds were discovered in the following decades. However, shortly after the introduction, resistance began to emerge and in all known cases emergence of antimicrobial resistance has followed the introduction of new antimicrobial compounds (Levy, 1982). It has now become clear that antimicrobial resistance poses a threat to human and animal health and should be taken seriously (Aarestrup, 2004: WHO, 2001)

The use of antimicrobial drugs in any ecosystem may select for antimicrobial drug resistant bacteria (Witte, 1998; O'Brien, 2002). Antimicrobial drugs used for food animals are often administered in a way that increases resistance, eg, .by subtherapeutic dosage, mass treatment and long-term administration such as antimicrobial growth promoters or by using drugs added to food and water for prophylaxis (Mateu and Martin, 2001). By the use of antimicrobial drugs for food animals, commensal bacteria and bacteria pathogenic to humans become reservoirs of resistance genes which reach humans through the food chain (Mølbak, 2004).

Deductions from the epidemiology of foodborne infections, ecological studies, outbreak investigations, typing studies and direct epidemiological observations show that resistant bacteria are transferred from food animals to humans. In addition to transfer in the food chain, exchange of mobile genetic elements among commensal and pathogenic bacteria contributes to the emergence of drug resistance. There is growing evidence that this has measurable consequences for human public health. The consequences are related to reduced efficacy of early empirical

treatment, limitations in the choices for treatment after confirmed microbiological diagnosis, and finally a possible co-selection of virulence traits. Recent epidemiological studies have measured these consequences in terms of excess mortality associated with resistance, increased duration of illness, and increased risk of invasive illness or hospitalisation following infections with resistant *Salmonella* (Mølbak, 2004).

Several *Salmonella* serotypes are associated with drug resistance. During 1994 and 1995 in the United States, the six resistant serotypes that were reported were *S.* Derby, *S.* Typhimurium var. Copenhagen, S. Typhimurium, *S.* Agona, *S.* Choleraesuis and *S.* Hadar (WHO, 1998). The same isolates were the most prevalent resistant strains in 1998 (Fedorka-Cray, 1998).

In France, during 1994 and 1995, 1,881 bovine and 2,438 poultry isolates of *Salmonella* were tested for antimicrobial resistance. The prevalence of resistance was 67.3% for the bovine isolates and 33.6% for the poultry isolates. Among the mono-drug resistant and multiple-drug resistant *Salmonella* eight resistant serotypes were found. These serotypes were *S.* Typhimurium, *S.* Enteritidis, *S.* Virchow, *S.* Newport, *S.* Hadar, *S.* Saintpaul, *S.* Montevideo, *S.* Infantis and *S.* Regent. Serotype *S.* Typhimurium, the most prevalent antimicrobial resistant serotype among the bovine isolates, represented 94% of all bovine *Salmonella* isolates that were resistant (WHO, 1998).

During period of 2000-2002, the National Veterinary Reference Laboratory for *Salmonella* (NRL-Salm) in Germany typed 11,911 isolates from animals, food, feed and the environment. All of them were tested for their susceptibility to 17 antimicrobial agents. Sixty-three per cent of all isolates were resistant and 40%were multiple resistant. This general resistance level was strongly influenced by those specific serotypes which dominate the *Salmonella* epidemiology in Germany. *S.* Typhimurium DT104 isolates from pig and cattle, and their resulting food products, were multiresistant in 98 and 94% of the cases respectively (Schroeter *et al*., 2004)

Multiple drug resistant *S.* Typhimurium DT104 was the second most prevalent *Salmonella* serotype isolated in humans in England and Wales and is increasingly prevalent in the United States and Canada. Outbreaks of multidrug resistant *S* Typhimurium DT104 have also been

reported in poultry, beef, cheese, and swine in numerous countries (Glynn *et al*., 1998; Cody *et al.*, 1999). This strain is resistant to a core group of antimicrobials, including ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline (commonly abbreviated ACSSuT). However, isolates have been identified which are also resistant to fluoroquinolones, trimethoprim, and kanamycin (Boyd *et al*., 2001).

Although the penta- or quadra-resistant pattern for these isolates is most often associated with the DT104 or DT104 complex phage types, other phage types are also observed. The most common phage type for *S.* Typhimurium other than DT104 appears to be DT193 while the most common phage type for *S.* Typhimurium var. Copenhagen is U302, which is similar to DT104. Infections associated with multidrug resistant *S.* Typhimurium DT104 have been associated with higher rates of admission to hospitals and mortality than other salmonellae. In addition, a study involving a small number of infections with multidrug resistant *S.* Typhimurium DT104 demonstrated a higher number of blood infections compared to those with sensitive strains (Boyd *et al*., 2001).

2.7. *Salmonella* in Ethiopia

2.7.1. *Salmonella* serotypes

Since the work of Pegram *et al*. (1981) who recovered 27 *Salmonella* serotypes from livestock and livestock products in Ethiopia, a number of researches undertaken revealed the presence of a large variety of *Salmonella* serotypes in the country.

Molomo (1998) isolated 10 *Salmonella* isolates belonging to *S.* Enteritidis *S.* Anatum and *S.* Uganda form poultry and poultry farm environment. Nyeleti *et al.* (2000) recovered *Salmonella* from 27.7% samples obtained from 235 cattle slaughtered at Addis Ababa abattoir. The major serotypes of the study include *S.* Anatum. *S.* Dublin and *S.* Muenchen. In another study conducted on cattle in Debre Zeit (Alemayehu *et al.*, 2003), 7% of the samples obtained from 323 animals were positive for *Salmonella*. *S.* Mishmarhaemek was the predominant serotype, which accounted for 52% of the total *Salmonella* isolates recovered. So far more than 60 *Salmonella*

serotypes were recovered by a number of other workers in Ethiopia from animals (Molla 2004; Molla *et al*., 2004; Aragaw, 2005; Demissie, 2005; Woldemariam *et al*., 2005; Molla *et al*., 2006), animal products (Tibaijuka *et al*., 2003; Molla and Mesfin, 2003; Ejeta *et al*., 2004; Zewdu, 2004) and humans (Gedebou and Tassew, 1981; Ashenafi and Gedebou, 1985; Mache *et al*., 1997, Nyeleti *et al*., 2000; Mache *et al*., 2002; Zewdu, 2004; Demissie, 2005). *S*. Typhimurium, *S*. Dublin, *S*. Typhi, *S*. Enteritidis, *S*. Infantis, *S*. Anatum, *S*. Braenderup *S*. Saintpaul, *S*. Hadar, *S*. Havana and *S*. Kottobus were isolated more frequently than the others.

2.7.2. Antimicrobial resistance

In developing countries like Ethiopia the situation of antimicrobial resistance is more complex and difficult. This is because *Salmonella* and other major zoonotic bacterial pathogens are not routinely cultured and their resistance to commonly employed antimicrobials both in public health and veterinary practice is rarely determined (Molla *et al*., 2003).

Studies conducted in Ethiopia on *Salmonella* in humans, animals, and animal products (Table 2 and 3) indicated the presence of a number of serotypes that are resistant to the commonly used antimicrobials (Mache, 2002; Alemayehu *et al*., 2003; Molla *et al*., 2003; Molla *et al*., 2004; Molla, 2004; Zewdu, 2004; Aragaw, 2005). Antimicrobial resistance studies conducted on human *Salmonella* isolates in Ethiopia (Table 2) show that the prevalence of resistance to one or more antimicrobial agents is high. Gedebou and Tassew (1981) reported 79.4% resistance to one or more drugs during 1975 to 1980 from diarrhoeal patients from various hospitals in Addis Ababa.

A more recent study conducted by Molla *et al*. (2006) on *Salmonella* isolates from pigs, showed that multiple antimicrobial resistance was more common than monodrug resistance. Of 94 *Salmonella* isolates recovered from 501 different samples of 101 pigs, 69 (73.4%) were resistant to two or more antimicrobials. Among the *Salmonella* serotypes a high level of MDR was observed in *S*. Hadar, *S*. Kentucky, *S*. Blockley and *S*. Enteritidis.

Table 2. Status and pattern of *Salmonella* antimicrobial resistance in humans in Ethiopia

Year	Location	No. Samples	Isolates Tested‡ No.	Resistant No. (%)	Multiple Resistance No. (%)	Common Serogroup(s) Isolated (No.)	Common Resistance Pattern (No.)	Maximum number antimicrobials showing resistance	References
1975-1980	Addis Ababa*	NA	165	131(79.4)	68(51.9)	Serogroup D (95)	SUL, STR*** (38)	10	Gedebou and Tassew (1981)
1982/1983	Addis Ababa*	1000	45	14(31)	10(71.4)	Serogroup C (10)	AMP, CAR, CEP, CHL KAN, STR, SUL, TET	8	Ashenafi and Gedebou (1985)
1995	Addis Ababa*	700	45	31(68.9)	29(93.5)	Serogroup C	AMP, CEP, SXT, TET, KAN (3) AMP, CEP, KAN, TET, CHL, SXT (3)	8	Mache *et al*. (1997)
2000	Jimma*	384	59	55(93.22)	51(92.7)	Serogroup B (15) Serogroup C (12) Typhi (11)	CEP, CHL, TET (4)	6	Mache (2002)
2004/2005	Debre Zeit	384	13	2 (15.4)	2 (100)	Enteritidis (1) Typhimurium var Copenhagen (1)	CIP, NAL, NIT (1) AMP, AMC, CHL, FLO, NIT, SPT, STR, SUL, TET (1)	9	Demissie (2005)

*Specimens from clinical cases. ‡ = All isolates were tested. NA = data not available.

***AMP = ampicillin, AMC = amoxicillin-clavulinic acid, CAR = carboncillin, CEP = cephalotin, CHL = chloramphenicol, CIP = ciprofloxcacin, FEN = fenicol, KAN = kanamycin, NIT = Nitrofurantion, SMX = sulfamethoxazole, SPC = spectinomycin, STR = streptomycin, SUL = sulfisoxazole, SXT = sulfamethoxazole/trimethoprim, TET = tetracycline, TIC = ticaccillin TMP = trimethoprim

Table 3. Status and pattern of *Salmonella* drug resistance in animals and animal products in Ethiopia

Year	Location	Species	No. Samples	No. of Isolates Tested‡	Resistant No. (%)	Multiple Resistance No. (%)	Most Common Serotype(s) Isolated (No.)	Common Resistance Pattern (No.)	Maximum Antimicrobials Resisted (No.)	References
1999/ 2000	Debre Zeit	Bovine **	1290	25	13 (52.0)	13 (100)	Mishmarhaemek (12)	AMP SMX, TIC (11)	6	Alemayehu *et al*. (2003)
2000/ 2001	Addis Ababa	Chicken **	301	54	31(68.9)	17(57.4)	Braenderup (10)	TMP, STR, SMX, SPC, TET	5	Tibaijuka *et al*. (2002)
2001/ 2002	Debre Zeit Addis Ababa	Chicken **	378	80	51(63.7)	42(82.5)	Typhimurium var. Copenhagen. (24) Braenderup (12)	AMP AMC, TET, CHL, FEN, SPC, SMX (18)	8	Molla *et al*. (2003)
2001/ 2002	Dire-Dawa, Jijiga	Camel **	714	116	51(44.0)	39(76.5)	Braenderup (23) Typhimurium (5)	AMP, STR, SXT, SMX, TMP, SPC (19)	8	Molla *et al*. (2004)
2003/ 2004	Addis Ababa	Sheep And Goats**	600	22	7(31.8)	7(100)	Typhimurium. (9)	AMP, CEP (3)	9	Molla (2004)
2003/ 2004	Addis Ababa	Food items	1268	98	32(32.7)	23(71.9)	Braenderup (10) Haifa (6) Kentucky (3) Hadar (3)	AMP, STR, SPC, SUL, SXT, TMP (10)	10	Zewdu, 2004
2004/ 2005	Addis Ababa	Swine**	849	173	57(32.9)	57(100)	Hadar (47)	NIT, STR, TET (44)	10	Aragaw 2005

** Samples from apparently healthy slaughtered animals

3. MATERIALS AND METHODS

3.1. Study area

The study was undertaken at a commercial slaughterhouse at Debre Zeit, Ethiopia. Debre Zeit is located 47 km Southeast of Addis Ababa at an altitude of about 1900 m.a.s.l. It receives an annual rainfall of 1115.6 mm with two rainy seasons. The short rainy season extends from March to May, while the main rainy season extends from June to September. The average maximum and minimum temperatures are 30.5^{o}C and 8.5^{o}C, respectively (NMSA, 2003). Bacteriological analysis of the samples was conducted at the Microbiology Laboratory of Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

In the slaughterhouse 500 to 1500 small ruminants mainly goats are slaughtered every day based on the demand from their importers in Saudi Arabia and United Arab Emirates. On average 35 male adult cattle were slaughtered for local market every Tuesday and Saturday regularly. Unscheduled slaughters, which sometimes may include small group of calves, could be conducted on other week days based on the demand from their major customers, which include among the others, student cafeterias of the Addis Ababa University, Civil Service College, prisons in Addis Ababa, a number of supermarkets and Sheraton Addis Hotel.

The slaughterhouse has one slaughter hall with two overhead rails. The rail for small ruminants is lower for operations to be carried out at the level of human height. The other rail is higher and serves cattle slaughter operations, where personnel stand on platforms to undertake most of the activities. Both the rails have their own entrances from outside for their respective slaughter animals. Cattle were slaughtered after small ruminants were completed and the slaughter hall floor was washed with tap water with hand held hoses. The same personnel were involved in slaughtering both groups of animals.

The slaughtering process involved stunning and hoisting on to the overhead rail, bleeding and front leg removal, hind leg removal followed by de-hiding, splitting of the sternum, evisceration, carcass splitting, weighing and chilling. Carcasses were pushed to the next process by the last person to complete the last operation. Hand washing was performed using

tap water after pushing the carcass to the next operation. Carcass washing was conducted only when accidental spillage of gut contents occurred during evisceration. The carcass was delivered after cutting it into quarters or in other cases different cuts and minced beef were delivered for their customers after 12 hours in chilling room.

3.2. Study animals

The study was conducted on apparently healthy animals slaughtered at a commercial slaughterhouse in Debre Zeit between October 2005 and February 2006. The cattle slaughtered in the slaughterhouse originated mainly from markets around Awash Park in the Rift Valley area of Afar and Oromia regions. Animals also originated from other parts of Oromia and were transported on double decked trucks made for animal transportation or on open trucks made for transportation of goods. After arriving at the slaughterhouse, 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 into another lairage where they spend the night, feed being withheld since separation from other animals.

3.3 Study design

The study was a cross-sectional observational study on 100 cattle slaughtered from October 2005 to February 2006. Animals were selected with systematic random sampling using the animal's slaughter order. On each visit 5 to 7 animals and equal number of holding pen samples were collected.

The variable of interest considered as an output variable at the slaughterhouse was carcass *Salmonella* status. The explanatory variables considered were *Salmonella* status of animal hide, flyer hands, eviscerator's hands, rumen content, caecal content, and mesenteric lymph nodes.

3.4. Sampling

The sample size required for the study was determined based on the expected prevalence of *Salmonella* and the desired absolute precision following Thrusfield (1995) by the following formula:

$$n = \frac{1.96^2 P_{exp}(1-P_{exp})}{d^2}$$

Where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

A previous study on *Salmonella* in an abattoir at the Faculty of Veterinary Medicine, Debre Zeit, Ethiopia revealed prevalence of 7% (Alemayehu *et al*., 2003). Therefore using the 7% expected prevalence, 95% confidence interval and 5% absolute precision the number of animals needed to demonstrate the prevalence of *Salmonella* was estimated to be 100 animals.

3.4.1. Sampling procedures

Sample animals were selected randomly and systematically, depending on the number of animals slaughtered on each day, a number was drawn to fix the beginning of the sample and then following their slaughter order. Hide (skin) swabs, rumen content, caecal content, mesenteric lymph nodes and carcass swab samples from each selected slaughtered cattle and corresponding flayer's hands and eviscerator's hand swab samples were collected in separate sterile sample containers. The samples were taken following slaughter line operations (Table 4). Selected animals were identified using two similar numbers attached by safety pin on the front leg after it was removed. One of these numbers was transferred to the eviscerated abdominal organs to match them with the carcasses. Holding pen swabs were collected from pens in which the animals spend the night after all the other samples were collected. The same type of swab was used for all samples, which required swabbing.

Hide swabs were taken immediately after the animals were stunned but before bleeding was carried out. Both sides of the animal and the median line that extended from the inguinal region to the neck were rubbed once from the posterior end to the anterior end of the animal using BPW (AES Laboratories, Cedex, France) moistened sterile sponge swabs of 80mm X 40mm size. The sponge swabs were moistened with 10ml of BPW at the time of collection. Swabbing of the hide was carried out by holding the swabs with sterile gloves. After swabbing, the swabs were returned to their original sterile plastic cases.

Samples from slaughter personnel hands (both flayer's and eviscerator's hands) were collected by rubbing the swabs on their both hands, inside and out, immediately after they completed their respective duties but before washing for the next one.

Rumen content samples were collected by cutting through the rumen wall using sterile scalpel blade for each sample. Solid contents were pushed to one side of the rumen and approximately 25ml of rumen fluid was decanted into sterile screw capped universal bottles. Approximately 25gm of caecal contents were collected by puncturing through caecal wall so that the contents were collected directly into sterile universal bottles.

The mesentery, with the lymph nodes attached, was removed from the surrounding structures using sterile scissors disinfected with 70% ethyl alcohol and brought to the laboratory in separate sterile plastic bags

Each carcass surface was sampled just before it entered into the chillier, by a previously described method (McEvoy *et al*., 2003). The entire outer surfaces of each carcass (both sides) were rubbed over once from hindquarter to the forequarters, uniformly using sterile sponge swab and a sterile pair of gloves.

Holding pen swabs were collected from 5 to 7 sites based on the number of holding pens used on that particular day. Approximately1m by 1m area of the holding pens were rubbed using BPW moistened swabs. The sample sites were constructed by drawing ideal grid lines between the vertical iron poles in either sides the fence of the pens.

Table 4. Type and number of samples collected from slaughterhouse at Debre Zeit

Type of sample	Number	Time of sampling
Hide swab (HS)	100	Before bleeding
Flayers hand swab (FH)	100	After de-hiding
Eviscerators' hand swab (EH)	100	After evisceration
Mesenteric lymph nodes (MLN)	100	After evisceration
Rumen content (RC)	100	After evisceration
Caecal content (CC)	100	After evisceration
Carcass swab (CS)	100	Before chilling
Holding swab sample (HPS)	100	At the end of sampling
Total	800	

Samples were transported in a cooler box on ice to the laboratory and were processed on the afternoon of the same day.

3.4.2. Sample processing

In the laboratory, 15 millilitres of BPW was added to each sample bag containing swab samples which were then manually agitated with fingers. The lymph nodes were aseptically freed from the surrounding tissue, and 25gm was weighed, immersed briefly in boiling water approximately for 10 seconds separately according to a previously described method (Moo *et al*., 1980). Each lymph node was then cut into smaller pieces on sterile petridishes by using sterile scalpel blade. The minced lymph nodes were put into sterile stomacher bags and 225 ml of BPW was added and homogenized for two minutes with stomacher (Seward Stomacher 400, London, UK) at high speed. Twenty-five grams of caecal content was weighed by directly transferring the content from the container into a stomacher bag near flame, and about 225 ml BPW was added. The resulting mixture was agitated using stomacher at low speed for 30 seconds. Twenty-five millilitres of rumen fluid was transferred from universal bottle to stomacher bag to which 225 ml of BPW was added and the mixture was manually agitated to disperse the contents. Whenever samples were less than 25 gm, BPW was added to the samples in 1:9 ratios.

3.5. Isolation and identification of *Salmonella*

Salmonella was identified and isolated according to standard techniques (ISO 6579, 1998; FDA, 1998; Quinn *et al*., 1999). The bacteriological media used in different stages were prepared according to the manufacturer's recommendations (Appendix I). Each sample was processed and analysed separately. ISO 6579 (1998) was used in this study because of the financial constraints for the purchase of other culture media such as MKTTn broth recommended by ISO 6579 (2002) and the presence of culture media in the laboratory bought according to the previous standard. According to ISO 6579 (2002) Muller-Kaufmann tetrathionate-novobiocin (MKTTn) and Rapaport-Vassiliadis soya (RVS) are recommended as selective enrichment broths while XLD is a primary agar medium and the second medium is of the user's choice like in ISO 6579 (1998).

3.5.1. Pre-enrichment

Processed samples in appropriate amount of BPW (1:9) were incubated for 16 to 20 hours at 37^{o}C. Mesenteric lymph node, rumen content and caecal content samples were incubated while they were in stomacher bags whereas all the swab samples were incubated in their original plastic bags.

3.5.2. Selective enrichment

Rappaport-Vassiliadis (RV) (Difco ^{TM}, Becton Dickinson, USA) and selenite cystine broth (SC) (Difco, Becton Dickinson, Claix, France) media were used for selective enrichment. One millilitre incubated pre enrichment broth was transferred aseptically into 10 ml of SC and was incubated at 37^{o}C for 18 to 24 hours. Another 0.1 ml of the pre-enrichment broth culture was transferred into 10 ml of RV broth and was incubated at 42^{o}C for 18 to 24 hours.

3.5.3. Selective plating and identification

A loopful of inoculum from each RV and SC cultures were streaked onto xylose lysine deoxycholate (XLD) agar (Oxoid, UK) and brilliant green phenol red lactose sucrose (BPLS) agar (Merck, Darmstadt, Germany) plates. The inoculated plates were incubated at 37^{o}C for

20 to 24 hours. After incubation, the plates were examined for the presence of presumptive *Salmonella* colonies. Typical colonies of *Salmonella* grown on XLD medium appear as pink colonies with or without black centre while on BPLS agar give an alkaline reaction and have red colonies. Many cultures of *Salmonella* on XLD medium, however, may produce colonies with large, glossy black centres or may appear as almost completely black colonies (ISO 6579, 1998; FDA, 1998; Quinn *et al.*, 1999).

3.5.4. Biochemical identification

For confirmation, up to five presumptive (typical or suspect) *Salmonella* colonies were picked from each selective plating media. When the suspected colonies on each plate were fewer than five, all the colonies were picked. Colonies were picked by lightly touching the centre of the colony with sterile inoculating needle.

Triple sugar iron agar (TSI) (Difco, Becton Dickinson, Claix, France) slants were inoculated by streaking slant and stabbing butt and without flaming, lysine iron agar (LIA) (Difco™, Becton Dickinson, Claix, France) slants were inoculated by stabbing butt twice and then streaking slant. TSI and LIA slants were incubated at 37°C for 24 ± 2 h with tubes capped loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. Isolates, which appear contaminated on TSI slant, were streaked onto XLD agar for purification. Pure isolates were inoculated on urea (BBL®, Becton Dickinson, USA) and Simmon's citrate agar (Difco, Becton Dickinson, Claix, France) slants, and Rambach® agar (Merck, Darmstadt, Germany) plates for further characterization. The inoculated Rambach® agar plates were incubated at 37°C for 24 hours (Anonymous, 1992) while urea agar slants were incubated for 24 h at 37°C (ISO 6579, 1998). Isolates presumptive of *Salmonella* on the biochemical tests were cultured on brain heart infusion agar (Difco, Becton Dickinson, Claix, France). One isolate from each positive sample was sent to Agence Française de Securite Sanitaire des Aliments (AFSSA), Unité, Caractérisation et Epidémiologie Bactérienne, Maisons-Alfort, Cedex, France for serotyping.

3.5.5. *Salmonella* serotyping

Serotyping of the *Salmonella* isolates was carried out at the Agence Française de Securite Sanitaire des Aliments (AFSSA), Unité, Caractérisation et Epidémiologie Bactérienne, Maisons-Alfort, Cedex, France. For serotyping, the somatic (O) antigens of the *Salmonella* isolates were determined with slide agglutination test as described by Ewing (1986), whereas the flagellar (H) antigens were identified by using a microtechnique (Shipp and Rowe, 1980) that employs microtitre plates. The antigenic formulae of *Salmonella* serotypes as listed by Popoff and Le Minor (1997) were used to name the serotypes.

3.5.6. Resistance to antimicrobial agents

Isolates biochemically and serologically confirmed as *Salmonella* were tested for their resistance to individual antimicrobial drug by the disk diffusion technique (NCCLS, 1997). Four to five well-isolated colonies grown on tryptic soy agar were touched by a disposable plastic loop and transferred to tubes containing 5ml of brain heart infusion broth. The broth culture was allowed to incubate at 35^{o}C for 4hrs until it achieves or exceeds the 0.5 McFarland turbidity standard (Appendix II). For those tubes, which exceeded the turbidity standard adjustments were made by adding sterile saline to obtain a turbidity visually comparable to the standard.

Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile swab was immersed in the dilution suspension and swabbed over the surface of two plates of Muller Hinton agar (BBL®, Becton Dickinson, USA) uniformly. The plates were held at room temperature for 10 min to allow drying. Using sterile forceps, disks impregnated with known concentration of antimicrobials were dispensed onto the surface of Muller Hinton agar plates. The type and concentration of antimicrobials in the discs are given in Table 5. The plates were incubated at 37^{o}C for 18 to 24hrs and examined for zones of inhibition. The diameters of the zones of inhibition were recorded to the nearest millimetre, and classified as resistant, susceptible or intermediate according to published interpretive chart (NCCLS, 1997). A standard reference strain of *E. coli* (ATCC25922), which is sensitive to all these drugs, was used for a quality control.

Table 5. Antimicrobials and concentrations used to test susceptibility of *Salmonella* isolates

Antimicrobial agent	Disk content (μg)	Zone diameter (mm)		
		Susceptible	Intermediate	Resistant
Amoxicillin/clavulanic acid	20/10	≥ 18	14-17	≤ 13
Tetracycline	30	≥ 19	15-18	≤ 14
Streptomycin	10	≥ 15	12-14	≤ 11
Sulfamethoxazole/Trimethoprim	1.25/23.75	≥ 16	11-15	≤ 10
Chloramphenicol	30	≥ 18	13-17	≤ 12
Kanamycin	30	≥ 18	14-17	≤ 13
Gentamicin	10	≥ 15	13-14	≤ 12
Neomycin	10	≥ 15	13-14	≤ 12

3.4. Data management and analysis

The data were entered and managed in MS Excel. The analysis was conducted using Stata version 7 (Stata Corporation, 2001). Prevalence of *Salmonella* at a sample and animal level was expressed as percentage, with 95% confidence interval (CI), of total number of positive samples or animals to total number of samples or animals examined. An animal was considered positive when a caecal content and/or mesenteric lymph node sample was culture positive for *Salmonell*a.

The data were analysed by comparing proportions, using Pearson's chi-square or Fisher's exact test based on the number of observations. The difference in serotype distribution in the different variables was compared using Pearson's chi-square test. For association of risk factors considered in the abattoir with carcass contamination, logistic regression analysis was used. The explanatory variables considered (HS, FH, EH, RC, CC, MLN *Salmonella* status) were separately analysed to see their association with the outcome of the bacteriological status of the carcass.

4. RESULTS

This study was conducted on 100 apparently healthy slaughtered cattle at a commercial slaughterhouse in Debre Zeit from October 2005 to February 2006 with objectives of estimating the prevalence of salmonellae, identifying serotype distribution and antibiotic resistance, and find out some of the risk factors associated with carcass contamination at slaughter. Bacteriological examination was conducted on 100 samples of each of the following: hide swabs (HS), flayer's hand swabs (FH), evisceration personnel's hand swabs (EH), rumen content (RC), caecal content (CC), mesenteric lymph node (MLN), carcass swabs (CS) and lairage or holding pen swabs (HPS).

4.1. Prevalence of *Salmonella*

Out of a total of 100 animals examined for bacteriological status of *Salmonella,* 14 (14%) were positive. An animal was considered *Salmonella* positive when it was bacteriologically positive either for its CC and/or MLN. Hide, rumen, and carcass status were considered as indicators of contamination and were not used for the calculation of prevalence of *Salmonella* serotypes. Of the samples examined, 31 (31%) of the skin swabs, 19 (19%) rumen contents, 8 (8%) mesenteric lymph nodes, and 6 (6%) of caecal content samples, were *Salmonella* positive. Only 2 (2.0%) of 100 carcass swab samples were positive for *Salmonella*. Six (6%) flaying personnel hand swabs, 2 (2%) eviscerating personnel hand swabs and 12 (12%) holding pen samples were positive for *Salmonella* (Table 6).

Table 6. Prevalence of *Salmonella* from different samples from slaughter cattle and environment

Sample	Number of samples		95% Confidence Intervals
	Examined	Positive (%)	
Hide swab	100	31 (31)*	22.1-41.0
Flayer hand swab	100	7 (7)*	2.9-13.9
Eviscerator hand swab	100	2 (2)	0.2-7.0
Rumen content	100	19 (19)	11.8-28.1
Caecal content	100	6 (6)	2.2-12.6
Mesenteric lymph nodes	100	8 (8)	3.5-15.2
Carcass	100	2 (2)	0.2-7.0
Holding pen	100	12 (12)	6.4-20.0
Total	800	87 (10.9)	8.6-13.2

*Significant, one - sided Fisher's exact = 0.03

No significant associations could be demonstrated between any of the variables and the carcass ($P > 0.05$). This was also demonstrated by the difference in the type of serotypes isolated. None of the major isolates from the other sample types were isolated from the carcass (Table 7).

There was however a significant difference in the overall distribution of serotypes between hide and flayer's hands (one-sided Fisher's exact = 0.03). There was a significant association ($P <0.05$) between hide contamination and flayer's hand contamination and the Odds ratio (OR) was 6.44. Flayer hands which came in contact with *Salmonella* positive hides were 6.44 times (OR = 6.44, 95% CI 1.1 - 35.3) more likely to be contaminated with *Salmonella* as compared to those who contacted negative hides.

Table 7. Distribution of *Salmonella* serotypes by sample type

Sample type and number of serotypes identified by source										
Serotype	HS	FH	EH	RC	CC	MLN	CS	HPS	Total	%
S. Anatum	24 [a, b]	5 [a]	1	12 [b]	2	2	-	9	55	63.2
S. Newport	4	2	1	5	2	1	-	3	18	20.7
S. Reading	-	-	-	-	-	5	-	-	5	5.7
S. Eastbourne	2	-	-	-	-	-	1	-	3	3.4
S. II 40:b: -	-	-	-	1	1	-	-	-	2	2.3
S. Bredeney	1	-	-	-	-	-	-	-	1	1.1
S. Typhimurium	-	-	-	-	1	-	-	-	1	1.1
S. Uganda	-	-	-	1	-	-	-	-	1	1.1
S. Urbana	-	-	-	-	-	-	1	-	1	1.1
Total	31	7	2	19	6	8	2	12	87	100

Distribution was significantly different between cells with similar letters (a, one-sided Fisher's exact = 0.01; b, Pearson's $\chi 2 = 5.05$, P = 0.025).

4.2. Distribution of serotypes

Among the 87 isolates of *Salmonella* recovered during the study (Table 7), 85 (97.7%) were specific serotypes of *Salmonella enterica* subsp. *enterica*. The rest 2 (2.3%) were *Salmonella enterica* subsp. *salamae* isolates. The two most frequently isolated serotypes were *S.* Anatum (63.2%) and *S.* Newport (20.7%). Serotypes recovered less frequently include *S.* Reading (5.7%), *S.* Eastbourne (3.4%), *S.* II 40:b:-(2.3%), and serotypes, Bredeney, Typhimurium, Uganda and Urbana (1.1% each).

There was a significant difference in the distribution of *S.* Anatum between hide and rumen content (Pearson's $\chi 2 = 5.05$, P = 0.025) and between hide and flayer's hands (one-sided Fisher's exact = 0.01). *S.* Anatum was more prevalent on the hide than in the rumen and on flayer's hands. Distribution of *S.* Newport was not significantly different (P > 0.05) between the different sample sources.

The predominant serotype in this study, *S.* Anatum, accounted for, 77.4, 71.4, 50, 63.1, and 75% of all the isolates from hide, flayers hands, eviscerator's hands, rumen contents and holding pen respectively. The respective prevalence of 12.9, 25.6, 50, 26.3, 33.3, and 25% of all the isolates in the same samples were *S.* Newport. Only these two serotypes were isolated from flayer's and eviscerator's hands, and holding pen swab samples. None of these serotypes were isolated from carcass swabs.

A different trend was noted for mesenteric lymph node samples, where *S.* Reading was the predominant serotype, accounting for 62.5% of the total isolates followed by *S.* Anatum and *S.* Newport which accounted for the rest 25% and 12.5%, respectively. All *S.* Reading isolates were recovered from mesenteric lymph nodes. *S.* Eastbourne isolates were recovered from hide and carcass swabs on the same day from different animals (Table 8). A maximum of two different serotypes were isolated from individual slaughtered cattle. The same serotypes were isolated from different samples of 10 animals, while 5 cattle were found to have two different serotypes in their different samples (Table 9).

Table 8. Serotype distribution in samples of carcass positive animals

Animal IDNo.	Sampling date	Serotype from carcass	Serotype from other related samples	Same serotype from other animals*
01	18/10/2005	*S.* Urbana	None	None
23	15/11/2005	*S.* Eastbourne	*S.* Newport	Present

*presence of similar serotype in samples collected from other cattle slaughtered on the same date

None of the two carcasses had similar serotypes isolated from other samples corresponding to them. In case of animal No. 23, similar serotype was isolated from skin of animal slaughtered on the same date (Table 8).

Table 9. Multiple serotypes isolated from different samples of individual slaughter cattle in Debre Zeit

Animal ID No	HS	RC	CC	MLN	CS
23	-	*S.* Newport	-	-	*S.* Eastbourne
24	*S.* Newport	*S.* Newport	-	-	-
28	-	*S.* Newport	-	*S.* Reading	-
34	*S.* Eastbourne	-	-	*S.* Reading	-
43	-	*S.* Newport	*S.* Newport	-	-
44	*S.* Anatum	*S.* Anatum	-	-	-
47	*S.* Bredeney	*S.* Anatum	-	-	-
48	*S.* Anatum	-	-	*S.* Anatum	-
49	-	*S.* Anatum	*S.* Anatum	-	-
51	-	*S.* Anatum	*S.* II 40:b:-	-	-
54	*S.* Anatum	*S.* Anatum	-	-	-
56	*S.* Anatum	*S.* Anatum	-	-	-
59	*S.* Anatum	*S.* Anatum	-	-	-
63	*S.* Anatum	*S.* Anatum	-	-	-
82	*S.* Anatum	*S.* Anatum			

4.3. Antimicrobial resistance

All of the isolates obtained during the study (n = 87) were tested for eight different antimicrobials that were available in the local market. Thirty-six (41.4%) of the isolates were resistant to one or more antimicrobials.

Out of the 9 serotypes, 5 showed resistance to various antimicrobials. Resistant isolates belong to the serotypes; *S.* Anatum, *S.* Newport, *S.* II 40:b:-, and *S.* Uganda. All other isolates were sensitive to all antimicrobials tested. These serotypes include *S.* Reading, *S.* Bredeney, and *S.* Typhimurium. However, 3 (n = 3/5) isolates of *S.* Reading and 1 (n = 1/1) isolate of *S.* Bredeney showed intermediate resistance to streptomycin.

Salmonella Anatum, the predominant serotype, was resistant mainly to one antimicrobial drug except one isolate which was resistant to two antimicrobials. Fourteen (25.5%) isolates of this serotype were resistant to streptomycin while 39 (69.1%) isolates demonstrated intermediate resistance to streptomycin. Only 3 (5.4%) isolates were fully sensitive to all antimicrobials tested in this study.

Table 10. Antimicrobial resistance patterns of *Salmonella* isolated from slaughtered cattle at Debre Zeit

	No. of isolates		
Serotype	Tested	Resistant	Antimicrobial resistance pattern
S. Anatum	55	14 (39*)	Str** (13)
			AmcStr (1)
S. Newport	18	18 (1*)	Tet (15)
			StrTet (2)
			AmcStrSxtTet (1)
S. Reading	5	(3*)	
S. Eastbourne	3	1	Str
S. II 40:b:-	2	2	Str (2)
S. Bredeney	1	(1*)	
S. Typhimurium	1	-	
S. Uganda	1	1	Str
S. Urbana	1	-	
Total	87	36	

(*) number of isolates showing intermediate resistance to streptomycin
** Amc, Amoxicillin/clavulanic acid; Sxt, sulfamethoxazole/trimethiprim; Str, streptomycin; Tet, tetracycline

All the isolates belonging to *S*. Newport in this study (n = 18) were resistant to tetracycline. Three (16.7%) of the isolates demonstrated resistance to one or more antimicrobials in addition to tetracycline. Other serotypes including *S*. II 40:b:- and *S*. Uganda were resistant to streptomycin (Table 10).

Five antimicrobial resistance patterns were identified (Table 10). The resistance ranges from 1 to up to 4 antimicrobials. The most common resistance pattern was for streptomycin. Only one isolate has a resistance pattern that extended beyond two antimicrobials (Table 10).

Among the antimicrobials against which *Salmonella* isolates were tested, the most frequent resistance was encountered for streptomycin. Twenty-one (24.1%) isolates were resistant to streptomycin, while 18 (20.6%) and 2 (2.3%) demonstrated resistance to tetracycline and amoxicillin/clavulanic acid respectively. Resistance to streptomycin was observed in *S.* Anatum, *S.* Newport, *S.* II 40:b:- *S.* Eastbourne and *S.* Uganda. Only isolates belonging to *S.* Newport showed resistance to tetracycline and sulfamethoxazole/trimethoprim. Resistance to amoxcicillin/clavulanic acid was exhibited by isolates of *S.* Anatum and *S.* Newport. All isolates were sensitive to chloramphenicol, gentamicin, kanamycin and neomycin.

Without considering the serotype composition and number of isolates recovered fro each sample, only serotypes recovered from carcass swab were found to be susceptible to all antimicrobial antimicrobials in the panel (Table 11).

Table 11. Distribution of antimicrobial resistance by sample source

Sample type	No. Examined	Serotype	No. Tested	No. Resistant
Hide	100	*S.* Anatum	24	7
		S. Newport	4	4
		S. Eastbourne	2	1
		S. Bredeney	1	-
Flayer hands	100	*S.* Anatum	5	1
		S. Newport	2	2
Eviscerator hands	100	*S.* Anatum	1	1
		S. Newport	1	1
Rumen content	100	*S.* Anatum	12	2
		S. Newport	5	5
		S. Uganda	1	1
		S. II 40:b:-	1	1
Caecal content	100	*S.* Anatum	2	1
		S. Newport	2	2
		S. Typhimurium	1	-
		S. II 40:b:-	1	1
Mesenteric lymph nodes	100	*S.* Reading	5	-
		S. Anatum	2	-
		S. Newport	1	1
Carcass	100	*S.* Eastbourne	1	-
		S. Urbana	1	-
Holding pen	100	*S.* Anatum	9	2
		S. Newport	3	3

5. DISCUSSION

5.1. Prevalence

In the present study, a high level of *Salmonella* prevalence was observed; 14% of the cattle carried *Salmonella* in their caecal contents or mesenteric lymph nodes. Such high prevalence was observed in an abattoir in Brisbane, Australia, where the prevalence in adult cattle was 14% for cows and 10% for steers (Daleel and Frost, 1967). However the prevalence we found was higher compared to a 4.3% in Smithfield, Australia (Nazer and Osborne, 1976). It was also higher than the findings of Nyeleti *et al*. (2000) and Alemayehu *et al*. (2003), which ranged from 0.9% to 3.8% in cattle slaughtered at Addis Ababa abattoir and the Faculty of Veterinary Medicine's small slaughterhouse in Debre Zeit, respectively. This could be as a 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 faecal excretion by carrier animals (Watson, 1975; Venter *et al*., 1994)

A 31% *Salmonella* prevalence from hide in this study was similar to the reports of Puyalto *et al*. (1997) who recovered *Salmonella* from 29 and 31% of hair and ears of cattle, respectively. It was however lower than the overall 68% prevalence reported by Fegan *et al*. (2005) in Australia, although there was a difference in *Salmonella* prevalence between the different groups of animals sampled by the investigators that ranged from 20 to 100%. Our report was higher than the 15.5% overall prevalence from beef animal hides in eight slaughterhouses in the USA (Bacon *et al*., 2002) and 17.7% report from Southwest England (Reid *et al.*, 2002). The prevalence of *Salmonella* on the external surfaces of cattle upon entry into a slaughterhouse serves as an indication of contamination that potentially could be transferred to underlying, otherwise sterile carcass surfaces during the dehiding process (Bacon *et al*., 2002). The presence of *Salmonella* excreters in batches of animals in transit and passing through the lairage results in external contamination of themselves and others in contact (Watson, 1975). It was also noted that stress associated with marketing and transportation of animals may contribute to an increase in shedding, which, coupled with increased animal density, would facilitate animal-to-animal spread both internally and externally (Bacon *et al*., 2002). In one longitudinal study it was shown that the prevalence of *S*. Typhimurium on hair increased from 10% before transportation to 35% after transportation and to 45% just before

skinning (Puyalto *et al*., 1997). *Salmonella* may also contaminate the hide from the floor of the stunning area (Samuel *et al*., 1980; Puyalto *et al*., 1997; Motsoela *et al*., 2002).

Isolation of *Salmonella* from 9% of slaughterhouse personnel hands involved in flaying and evisceration was much less than the 44% overall prevalence of *Salmonella* from workers in a beef abattoir in Australia (Smeltzer and Collins, 1980a). In both studies however, the prevalence of *Salmonella* was higher in those personnel having direct contact with the hide. The lower prevalence in this slaughterhouse could be the result of frequent hand washing which might have reduced build up of bacteria on the hands of those personnel to undetectable levels.

Rumen prevalence of 19% of this study was in agreement with 25% prevalence of *Salmonella* from rumen of cattle slaughtered in Australia (Fegan *et al*., 2005). However it was much higher than the 2% report from an Irish commercial abattoir (McEvoy *et al*., 2003). Higher prevalence in this study might have been caused by the resumption of feeding after transportation to the slaughterhouse or due to cross contamination through feeding and watering troughs during 24 to 72 hours when the animals stayed in the lairage. Grau and Brownlie (1968) reported 36% prevalence of *Salmonella* in the rumen of cattle held for longer periods between farm and slaughter.

The prevalence of *Salmonella* in the caecal contents in this study was 6%. It was in agreement with the 6% prevalence of *Salmonella* for feedlot cattle (Fedorka-Cray, 1998) and 5% for dairy cows (Wells *et al*., 2001) in the USA. However, it was higher than previous reports of 2.1% prevalence from faecal cultures of cattle slaughtered in Addis Ababa abattoir (Pegram *et al*., 1981; Nyeleti *et al*., 2000). Another investigation in an abattoir in the Faculty of Veterinary Medicine (FVM) at Debre Zeit indicated that the prevalence of *Salmonella* from faeces could be as low as 0.6% (Alemayehu *et al*. 2003). The prevalence in our study was also higher than the 1% faecal prevalence observed for beef cows in the USA (Dargatz, 2000). The reason for the relative higher caecal prevalence of *Salmonell*a in this study could be associated with the exposure of these animals for transportation and longer lairage confinement. Exposure of animals to such predisposing factors as unhygienic conditions, overcrowding and transportation, leads to shedding of *Salmonella* by carrier animals (Venter *et al*., 1994). A study conducted by Morgan *et al*. (1987) to determine the effect of time spent in lairage on salmonellosis, showed that *Salmonella* serotypes were isolated from 18.5, 24.1

and 47.7% of pigs from the same shipment of one producer held in lairage for 24, 48 and 66 hours, respectively.

The 8% *Salmonella* prevalence in the mesenteric lymph nodes in this investigation was higher than previous reports of 2.1% (Pegram *et al*., 1981) and 0.9% (Alemayehu *et al*., 2003) from Addis Ababa abattoir and FVM's abattoir at Debre Zeit, respectively. However, our mesenteric lymph node prevalence was much lower than the 57.1% and 30% from two different commercial abattoirs in Australia reported by Samuel *et al*. (1980) and Moo *et al*. (1980), respectively. The relatively lower *Salmonella* prevalence in this study as compared to those from the latter two studies could be attributed to the differences in animal husbandry. Salmonellosis is more prevalent in areas with intensive animal husbandry where animals live in a confined environment and require frequent cleaning (Wray and Davies, 2003).

The prevalence of *Salmonella* from carcasses in our study was 2%. This was in agreement with the 2.8% and 3.1% *Salmonella* prevalence from abdominal muscles and diaphragmatic muscles, respectively from cattle slaughtered in a small abattoir at FVM, Debre Zeit (Alemayehu *et al*., 2003). It was however lower than the 9.8% abdominal muscle and 11.9% diaphragmatic muscle from cattle slaughtered at Addis Ababa abattoir (Nyeleti *et al*., 2000). It was also lower than the 7.6% report from The Republic of Ireland (McEvoy *et al*., 2003). This difference could be attributed to the differences in abattoir facilities, sampling techniques, number of animals slaughtered, and the level of hygiene maintained by the abattoirs. Our carcass prevalence of *Salmonella* was in agreement with 2% carcass contamination before chilling from an abattoir in Australia (Fegan *et al*., 2005) and 1.3% beef carcass contamination in USA (Bacon *et al*., 2002). The similarities and differences in carcass contamination levels have to be taken with caution because of the differences in which the studies were conducted. For example, the carcass contamination rates in the latter two studies were obtained after a number of decontamination measures were taken, to reduce the bacterial load of carcasses, while no decontamination measures were undertake in the current study, except in cases where there were accidental spillages of gut contents, during which it was washed with tap water by sprinkling with a rubber hose.

The presence of even small number of salmonellae in carcass meat and edible offal may lead to heavy contamination of minced meat and sausages (Watson, 1975). Therefore as the

slaughterhouse produces minced beef for a number of local supermarkets further amplification of *Salmonella* could occur, becoming a public health risk.

5.2. Serotypes

Eighty-five of the 87 isolates recovered during this study were identified as specific serotypes belonging to *Salmonella enterica* subspp *enterica*. The other two isolates belong to subspp *salamae.* Among the *Salmonella* isolates identified, the most frequently isolated serotype was *S.* Anatum accounting for 63.1% of the total isolates. This serotype was previously reported in Ethiopia from slaughtered cattle (Nyeleti *et al*., 2000), camel (Molla *et al*., 2004), goats (Woldemariam *et al*., 2005), and swine (Aragaw, 2005). It was also recovered from animal products such as minced beef (Nyeleti *et al*., 2000; Ejeta *et al*., 2004; Zewdu *et al*., 2004), mutton (Ejeta *et al*., 2004; Zewdu 2004) chicken meat and giblets (Tibaijuka *et al*., 2003; Molla and Mesfin, 2003; Zewdu, 2004) and stool specimens (Demissie, 2005). However, this was the first time that it was recovered in such a large proportion in the country. It was also reported by other investigators that *S.* Anatum was the second most commonly isolated serotypes in cattle of the USA (Wray and Davies, 2003). Fegan *et al*. (2005) were able to isolate *S.* Anatum from 7% of the oral cavities, 5.8% of hides, 4% of rumen, and 13.6% of holding pen faeces samples in a study conducted in one abattoir in Australia. Dargatz *et al*. (2003) reported *S.* Anatum as the most common isolate from feedlot cattle pen floor in the USA accounting for 27.4% of all the isolates. Motsoela *et al*. (2002) reported that 34.8% of *Salmonella* isolates from soil in corals and stunning area in two Gaborone abattoirs to be *S.* Anatum. It was the ninth frequently isolated nontyphoid *Salmonella* in humans in Taiwan in 1995 (Chiu *et al.*, 2004). This clearly indicates that the serotype is widely distributed both in Ethiopia and elsewhere.

The second most frequent serotype (20.7%) recovered in our study was *S.* Newport. Although this is the first time that it is reported from cattle in Ethiopia, it was previously reported from a variety of food items (Zewdu, 2004). Being the predominant isolate, it accounted for 13.8%, 54.5%, 52.2% 25%, 75%, 66.6%, and 100% of all the *Salmonella* isolates from chicken meat, pork, mutton, minced beef, local cheese, fish and stool samples obtained from supermarkets and their personnel in Addis Ababa, respectively (Zewdu, 2004). Aragaw (2005) recovered *S.* Newport from slaughtered swine, which accounted for 2.3% of the total *Salmonella* isolates from swine slaughtered at Addis Ababa abattoir. *S.* Newport was the fifth most common

Salmonella serotype in cattle in the UK during the year 2000 (MAFF, 2000) and USA during the period 1999-2000 (Wray and Davies, 2003). This serotype was previously reported in human foodborne gastroenteritis in the USA and Canada (D'Aoust, 1997). It was the third common laboratory confirmed *Salmonella* isolate from humans in USA in 1999 preceded only by *S.* Typhimurium and *S.* Enteritidis, and the seventh in Taiwan during 1991 to 1996 (Chiu *et al*., 2004). Food components such as meat that contain high fat content are associated with low infectious doses (D'Aoust, 1997). An outbreak of human salmonellosis due to *S.* Newport with an infectious dose of 10 to 100 salmonellae was reported in the USA (Fontaine *et al*., 1987). *Salmonella* Newport outbreaks reported to CDC in the USA are generally associated with the consumption of beef (Zhao *et al*., 2003). Therefore, the isolation of this serotype in such a proportion in this study, and its dominance in the previous study in food items coupled with the tradition of raw and/or undercooked meat consumption in the society, is of public health concern, as the organism may reach the consumer along the production chain from abattoir to consumers' table.

Salmonella Reading was the third most common isolate recovered. It constituted 5.7% of all the isolates in our study. The serotype was not previously reported from cattle in Ethiopia, however, it was reported from mesenteric lymph nodes and faeces of sheep slaughtered in Addis Ababa and Modjo abattoirs (Molla, 2004). All of the isolates of this serotype in the study were recovered from mesenteric lymph nodes of 5 cattle, suggesting that the animals might have been infected prior to their entry to the slaughterhouse. From public health point of view, *S.* Reading was rarely associated with beef. From 1973 through 1989, eight foodborne outbreaks of *S.* Reading infection were reported to CDC's Foodborne Disease Outbreak Surveillance System. Consumption of improperly cooked Turkey was implicated as the source of infection in six of these outbreaks. Another foodborne nocosomial outbreak of due to this serotype in Connecticut, USA, which affected hospital workers and patients, was also traced back to improperly cooked turkey in the hospital's cafeteria (CDC, 1991).

Salmonella Eastbourne accounted for 3.4% of the total *Salmonella* serotypes isolated in our study. No comparison can be made with other works, as there is no information from previous works in Ethiopian cattle. It was previously isolated as one of the major *Salmonella* isolates from slaughtered swine in Addis Ababa abattoir (Aragaw, 2005). Contrary to our finding this serotype was not among the top ten *Salmonella* isolates commonly reported from cattle in the USA (Wray and Davies, 2003), UK (MAFF, 2000) and the Netherlands (van Duijkeren,

2002). *S.* Eastbourne was reported from human outbreak of salmonellosis in the USA and Canada (D'Aoust, 1997).

Salmonella Typhimurium was one of the least frequently isolated serotype in our study. However, it was the most common isolate from clinical cases of cattle reported previously accounting for 50% of all the *Salmonella* isolates from clinical cases from cattle in and around Addis Ababa (Pegram *et al.*, 1981). It was also recovered from slaughtered cattle (Alemayehu *et al*., 2003), camel (Molla *et al*., 2004), sheep and goats (Molla, 2004; Woldemariam *et al*., 2005). *S.* Typhimurium was isolated from a variety of food items of animal origin including minced beef (Zewdu, 2004), chicken meat and giblets (Mesfin and Molla, 2003; Zewdu, 2004). It was also isolated previously from 1.9% of calves under six months of age from five smallholder dairy farms in Debre Zeit (Demissie, 2005). During the same period it was detected from 1.7% out-patients treated at Bishoftu Hospital and Bishoftu Health Centre, accounting for 46.2% of the total *Salmonella* serotypes from humans (Demissie, 2005). Contrary to our findings, *S.* Typhimurium is one of the most common *Salmonella* serotype isolated from cattle elsewhere (Smeltzer and Collins, 1980a; McEvoy *et al*., 2003). Isolation of *S.* Typhimurium in our study, though small in proportion, has major significance both for cattle and public health, as it is one of the predominant causes of salmonellosis in cattle and humans. *S.* Typhimurium was the most common *Salmonella* recovered from clinical infections in humans in the USA in 1999 and 2003 (CDC, 2003). It was also the most common isolate from humans in the Netherlands from 1984-2001 (van Duijkeren, 2002).

Salmonella Urbana, which was one of the two isolates recovered from carcasses, was not previously reported from animals and food of animal origin in Ethiopia. Pegram *et al*. (1981) reported *S.* Urbana from equipment in bone factory located outside of Addis Ababa. This serotype was not among the commonly reported serotypes in Ethiopia. The isolation of a single *S.* Urbana from a carcass and not from other samples could indicate the presence of other sources of contamination. Dissemination of *Salmonella* through steel with direct or indirect contact with carcasses has been described (Stolle, 1980; Smeltzer and Collins, 1980a and b). It was also possible that *Salmonella* might have spread from positive adjacent carcasses through close contact, which was the case during our study.

Salmonella Uganda accounted for 1.1% of the total isolates. It was previously reported from chicken and chicken products as the third most common isolate in that study accounting for

11.1% of the total *Salmonella* isolates (Tibaijuka *et al*., 2003). Molomo (1998) isolated 2 (2/10) *S*. Uganda isolates from dust in layer hen pens from a poultry farm in Debre Zeit. A more recent investigation on *Salmonella* contamination levels of minced beef collected from supermarkets in Addis Ababa, recovered *S*. Uganda as a predominant serotype accounting for 40% of the total isolates (Demissie, 2005).

Salmonella Bredeney was isolated as one of the least isolated serotypes, 1.1% of the total isolates. It was reported from slaughtered livestock in Addis Ababa abattoir (Pegram *et al*., 1981). It was not detected in other previous studies in Ethiopia indicating the rare occurrence of the serotype in Ethiopia. This serotype was not among the common *Salmonella* serotypes identified from any source (MAFF, 2000; Motsoela *et al*., 2002; Wray and Davies, 2003).

Salmonella Dublin, an important cause of bovine salmonellosis, which was previously reported in slaughtered cattle (Pegram *et al*., 1981; Nyeleti, *et al*., 2000; Alemayehu *et al*., 2003) and minced beef (Ejeta *et al*., 2004) in Ethiopia, was not recovered in our study.

No significant association ($P > 0.05$) could be demonstrated between the variables considered to be sources of contamination (hide, rumen content, caecal content, mesenteric lymph nodes, flayer's and eviscerator's hands) and bacterial status of the carcass. This was also demonstrated by *Salmonella* serotypes recovered from the carcasses. Both isolates recovered from the two carcasses, *S*. Eastbourne and *S*. Urbana were among the least recovered serotypes. *S*. Urbana was recovered only from a carcass. This was comparable to the results of Molla *et al*. (2006), where one of the two *Salmonella* isolates recovered from the two *Salmonella* positive carcasses, was not recovered from any one of the other four sample types.

5.3. Antimicrobial resistance

All of the isolates recovered during this study were tested against a panel of eight antimicrobials. Out of the 87 isolates tested, 36 (41.4%) were found to be resistant to one or more antimicrobial drugs. Our result compares favourably with 44.8% resistance reported for isolates from camel (Molla *et al*., 2004). It was however, higher than 32.7%, 31.8% and 31.9% overall resistance reported for isolates from food samples (Zewdu, 2004), slaughtered sheep and goats (Molla, 2004), and swine (Aragaw, 2005), respectively. Our result however was lower than the prevalence of antimicrobial resistance (57 to 64%) for one or more

antimicrobials reported from Ethiopian chicken carcass and giblets (Tibaijuka *et al*., 2002; Molla *et al*., 2003). A high level of antimicrobial resistance (93%) has also been observed for diarrhoeal out-patients (Mache *et al*., 1997; Mache, 2002) in Ethiopia. The differences observed in the current and previous studies on the level of antimicrobial resistance might be due to differences in the number of antimicrobial drugs tested in the present study as well as the diversity in their usage. These differences could also arise from the frequency and type of antimicrobials used in an area from which the animals originated.

Multiple antimicrobial resistance was observed in 4 (11.1%) of the 36 resistant isolates. This was by far lower than multiple drug resistance of *Salmonella* isolates reported from different sources in Ethiopia. Multiple drug resistance among the human antimicrobial resistant *Salmonella* isolates in Ethiopia, ranged from 51.9% (Gedebou and Tassew, 1981) to 93.5 % (Mache *et al*., 1997) with results from other studies fitting between these values (Ashenafi and Gedebou, 1985; Mache, 2002). Most of the reports by different investigators on antimicrobial resistance of salmonellae in slaughtered animals and animal products in Ethiopia, indicated that the proportion of multidrug resistant isolates was higher than that of monodrug resistant isolates, ranging from 54.9% (Tibaijuka *et al*., 2002) to 100% (Alemayehu *et al*., 2003; Molla, 2004; Aragaw, 2005). Reports by other investigators fall between these values (Molla *et al*., 2003; Zewdu, 2004; Molla *et al*., 2006).

Salmonlla Anatum was resistant predominantly to streptomycin except in one case where it was resistant to amoxcicillin-clavulanic acid combination in addition to streptomycin. Fourteen (25.5%) isolates were resistant to streptomycin, while 39 (70.9%) of the isolates showed intermediate resistance. A previous study by Molla *et al*. (2003) detected (n = 5/8) multiple antimicrobial resistant *S*. Anatum isolates resistant to up to 7 antimicrobials including streptomycin. It was in agreement that no *S*. Anatum isolate was resistant to tetracycline. *S*. Anatum previously isolated by Nyeleti *et al*. (2000), Molla *et al*. (2003) and Ejeta *et al*. (2004) were sensitive to all the antimicrobials tested.

Although the mechanism of resistance was not described, the apparent increase in the number of *Salmonella* isolates expressing intermediate resistance to streptomycin, led to the suggestion that resistance among *S. enterica* isolates to streptomycin develops in two successive stages consisting of reduced resistance followed by complete resistance to the drug

(Cormican *et al.*, 1998). McEvoy *et al*. (2003) have also reported 52% intermediate resistance to streptomycin among *Salmonell*a isolates tested. The apparent high intermediate resistance in this study implies large proportion of these isolates would become resistant to streptomycin over relatively shorter period of time in the future.

All *S*. Newport isolates in our study (n = 18) were resistant to tetracycline. Three (16.7%) of the isolates acquired resistance to one or more additional antimicrobials. Previous *S*. Newport isolates from swine (Aragaw, 2005), foods of animal origin (Zewdu, 2004), and human beings (Zewdu, 2004) in Ethiopia were susceptible to all the antimicrobials tested (including all the drugs used in our investigation). *S*. Newport is an emerging multiple resistant *Salmonell*a serotype in the USA both in humans (Dunne *et al*., 2000) and animals (Winokur *et al*., 2001; Rankin *et al*., 2002; Dargatz *et al*., 2003). Resistance to tetracycline in *Salmonella* isolates is conferred by a resistance gene encoded on resistance plasmids also known as R plasmids (Rankin *et al*., 2002). *Salmonella* are among those known to carry plasmids, which encode for drug resistance. This implies that widespread use of antimicrobials in animals and humans may cause an increase in the frequency of occurrence of bacteria resistant to one or more drugs encoded on the plasmids (Witte, 1998; Poppe *et al*., 2002; Mølbak, 2004). A number of investigators have shown the transfer of such resistance plasmids through conjugation to similar or even unrelated group of organisms in the presence of stress, in this case antimicrobials (Su *et al*., 2003; McCuddin *et al*., 2006) Therefore the emergence of antimicrobial resistance in this serotype over a short period of time in this study, as compared to the previous reports in Ethiopia, is of public health concern as meat and meat products contaminated with antimicrobial resistant *S*. Newport could reach large number of consumers through the production chain.

One (1/3) isolate of *S*. Eastbourne was resistant to streptomycin. No antimicrobial resistance was detected previously to this serotype (Pegram *et al*., 1981; Aragaw, 2005). A single isolate of *S*. Uganda recovered during the study was resistant to streptomycin. Intermediate resistance of the latter to other antimicrobials was previously reported by Demissie (2005). *S*. Bredeney showed intermediate resistance to streptomycin indicating its potential to become resistant to this drug. No resistance to any antimicrobial was reported when it was previously isolated in Ethiopia (Pegram *et al*., 1981). It was not among the commonly reported antimicrobial resistant serotypes elsewhere (Dargatz *et al*., 2002).

In this study no isolates belonging to *S*. Reading were fully resistant to any of the antimicrobials. However, Molla (2004) recovered 2 (2/7) multidrug resistant *S*. Reading isolates which were resistant to streptomycin, sulfamethoxazole and tetracycline from sheep and goats slaughtered at Addis Ababa and Modjo abattoirs. Resistance of *S*. Reading was reported from feedlot operations in the USA, where 95.7% of the isolates acquired resistance to one or more antimicrobials (Dargatz *et al*., 2002). In this study three (3/5) isolates of *S*. Reading showed intermediate resistance to streptomycin.

Salmonella Typhimurium and *S*. Urbana were sensitive to all the antimicrobials tested. This was in agreement with Demissie (2005), who tested *S*. Typhimurium isolates from dairy calves in Debre Zeit, and Pegram *et al*. (1981) who reported both serotypes to be susceptible to all the antimicrobials tested in their respective investigations. Contrary to our work, *S*. Typhimurium and/or Typhimurium var Copenhagen multiple resistant to up to 10 antimicrobials were recovered by a number of investigators (Molla *et al*., 2003; Molla, 2004; Molla *et al*., 2004, Aragaw, 2005). *Salmonella* Typhimurium DT104, resistant to a core group of antimicrobials, including ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline, was among those reported in Ethiopia (Molla *et al*., 2004)

In general streptomycin was the least effective antimicrobial. Out of the 87 total *Salmonella* isolates 21 (24.1 %) were resistant to streptomycin. Out of the 36 resistant isolates in the study 58.3 % (21/36) were resistant to streptomycin. This result was in agreement with Molla *et al*., (2003) who reported 22.5% resistance to streptomycin among the *Salmonella* isolates from chicken carcasses and giblets in Addis Ababa and Debre Zeit. Other investigators reported higher prevalence for streptomycin resistance from apparently healthy slaughtered animals and foods of animal origin (Tibaijuka *et al*., 2002; Molla, 2004; Zewdu, 2004; Molla *et al.*, 2004; Aragaw, 2005) and human out-patients (Gedebou and Tassew, 1981; Ashenafi and Gedebou, 1985). Streptomycin, alone or as a combination with other antimicrobials, was one of the most commonly used drug both in humans and animals in Ethiopia for a long period of time (Pegram *et al*., 1981; Gedebou and Tassew, 1981), and resistance to this drug in *Salmonella* serotypes was reported in humans in the late 1970's (Messele and Alebachew, 1979). Both overuse and under use through lack of access, inadequate dosing, poor adherence and substandard antimicrobials may play an important role in development of antimicrobial resistance (WHO, 2001).

Eighteen (20.7%) of the 87 *Salmonella* isolates were tetracycline resistant. This was comparable to 13.6% lower resistance to tetracycline from sheep and goats (Molla, 2004), and 15.3% from food items (Zewdu, 2004). On the other hand higher resistance, as compared to our result, was reported from human isolates in Ethiopia. More than 71% and 59.3% of isolates from human out-patients in Addis Ababa and Jimma were resistant, respectively (Mache *et al*., 1997; Mache, 2002). Higher levels of resistance have also been reported from animals and animal products in Ethiopia. Molla *et al*. (2003) reported a 41.2% resistance among isolates of *Salmonella* from chicken sources. Dargatz *et al*. (2003) reported 35.9% prevalence of *Salmonella* resistance to tetracycline among isolates recovered from pen floor of 73 feedlots in 12 states in the USA. Similar reasons given to possible causes of antimicrobial resistance to streptomycin holds for resistance to tetracycline, as it was the most commonly used drug in treatment of infectious diseases both in humans and animals in the country.

Resistance to amoxcicillin/clavulanic acid was observed in 2.3% of the isolates. This finding was comparable to 2.6% and 4% prevalence reported by Molla *et al.* (2004) and Aragaw (2005) in camels and swine, respectively. However, it was much lower than 25% and 45% resistance reports of Zewdu (2004) and Molla *et al*. (2003), foods and chicken carcasses and giblets, respectively Resistance to amoxicillin/clavulanic acid reported from a feedlot survey in the USA was 9.4%, which was higher than our observation (Dargatz *et al*., 2003). In another investigation in the USA, 45.3% (24/53) *Salmonella* positive samples from animal hide and carcasses yielded at least one isolate resistant to amoxcicillin/clavulanic acid (Bacon *et al*., 2002). Resistance to amoxicillin/clavulanic acid in this study might have been the result of drug usage in humans in Ethiopia as this drug have not been used in the treatment of animals.

Resistance against sulfamethoxazole/trimethoprim was 1.1%, which was in agreement with 0.6% prevalence reported by Aragaw (2005) and disagrees with a number of studies previously conducted in the country on isolates from humans and poultry products (Mache *et al*., 1997; Mache, 2002, Tibaijuka *et al*., 2002; Molla *et al*., 2003; Zewdu, 2004). This combination was not previously used in the country for treatment of diseases in cattle therefore resistance to sulfamethoxazole/trimethoprim might have been associated with the use of the drug in humans and poultry in different parts of the country. The use of antimicrobial drugs in one ecosystem was shown to select for antimicrobial drug resistant

bacteria within that ecosystem and the same resistance could then be detected in bacteria in different ecosystems (Witte, 1998; O'Brien, 2002). Alternatively resistance to this combination of drugs might have been developed separately to each individual drug in the combination.

Differences and similarities observed in the overall antimicrobial resistance between this work and works by previous investigators particularly, with those conducted in Ethiopia, should be cautiously interpreted, as there were differences in the number of antimicrobials used and the composition of resistant serotypes between this and previous studies, where there were similarities in their predominantly isolated resistant serotypes. In most of the previous investigations the predominant antimicrobial resistant *Salmonella* serotypes were *S*. Braenderup (Tibaijuka *et al*., 2002; Molla *et al*., 2003; Molla *et al*., 2004; Zewdu, 2004), *S*. Typhimurium and/or Typhimurium var Copenhagen (Molla *et al*., 2003; Molla *et al.*, 2004; Molla, 2004), *S*. Kentucky and *S*. Hadar (Zewdu, 2004; Aragaw, 2005; Molla *et al*., 2006). The two frequently isolated serotypes in our study were either not reported or when reported, they were found to be susceptible to all antimicrobial agents tested except in few studies, where *S*. Anatum was found to be multidrug resistant to two up to seven antimicrobials (Tibaijuka *et al*., 2002; Molla *et al*., 2003).

6. CONCLUSION AND RECOMMENDATIONS

In the present study a high level of *Salmonella* was detected in slaughtered cattle at a commercial slaughterhouse in Debre Zeit, Ethiopia. There was also high *Salmonella* contamination of the hides, rumen contents and flayer's hands. *Salmonella* was also isolated from eviscerator's hands. However, the carcass contamination as opposed to the entrance of animals with salmonellae on their hides, mesenteric lymph nodes and caecal contents into the slaughterhouse, was very low. This indicates slaughter processes followed by the slaughterhouse have reduced carcass contamination.

The similarity of the isolates between the caecal contents, the holding pens, the hides and the rumen contents indicates the source of contamination in the abattoir was most probably associated with supply of infected animals. However, the recovery of *S*. Urbana from the carcass and not from any one of the expected sources of contamination indicates the presence of other possible sources of contamination.

Salmonella Anatum was the predominant serotype identified in this study followed by *S*. Newport, *S*. Reading and *S*. Eastbourne. Other least frequently recovered isolates include, *S*. II 40:b:-, *S*. Bredeney, *S*. Uganda, *S*. Urbana and *S*. Typhimurium.

Antimicrobial resistance to one or more drugs was prevalent. Resistance was observed mainly to streptomycin and tetracycline followed by amoxicillin/clavulanic acid and sulfamethoxazole/trimethoprim. Of the *Salmonella* serotypes tested, a high level of resistance was observed for *S*. Newport and *S*. Anatum isolates.

Based on the above conclusions the following recommendations are forwarded:

- Further investigation has to be made to determine other sources of carcass contamination in the slaughterhouse to determine the measures that should be taken to reduce contamination from the other sources.

- The fact that *Salmonella* serotypes in this study were not the predominant serotypes reported in cattle in other studies and the isolation of relatively high number of

serotypes signify the need to undertake well-structured further surveys on the prevalence and distribution of *Salmonella* serotypes in Ethiopia in order to promote particularly, cattle and beef export in the country.

- Risk factors for antimicrobial resistance by *Salmonella* serotypes in Ethiopia have to be investigated.

- Antimicrobial resistance monitoring system and the prudent use of antimicrobials at all levels are needed to reduce and prevent the occurrence of high antimicrobial resistance in cattle and other food animals as well.

7. REFERENCES

Aarestrup, F. M. (2004): Monitoring of antimicrobial resistance among food animals: principles and limitations. *Journal of Veterinary Medicine Series B,* **51**, 380–388.

Acha, P. and Szyfres, B. (2001): Zoonoses and Communicable Diseases Common to Man and Animals; Bacterioses and mycoses. 3 rd ed. Volume I. Washington, D. C.: Pan American Health Organization. pp. 233-246.

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

Andrews, W. H. (1989): Methods for recovering injured "classical" enteric pathogenic bacteria (*Salmonella, Shigell*a, and enteropathogenic *Escherichia coli*) from foods. In: B. Ray (ed.), Injured Index and Pathogenic Bacteria, CRC Press, Boca Raton, FL. pp. 55-113.

Anonymous. (1992): The Merck Manual. Merck, Darmstadt, Germany.

Anonymous. (1990): The Oxoid Manual. Oxoid Ltd., Basingstoke, Hampshire, United Kingdom.

Anonymous. (1996): *Salmonella* in Livestock Production. London: Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory.

Aragaw, K. (2005): *Salmonella* in apparently healthy slaughtered swine in Addis Ababa, Ethiopia. Addis Ababa University, Faculty of Veterinary Medicine, MSc thesis.

Ashenafi, M. and Gedebou, M. (1985): *Salmonella* and *Shigella* in adult diarrhoea in Addis Ababa: prevalence and antibiograms. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **79**, 719-721.

Bacon, R. T., Sofos, J. N., Belk, K. E., Hyatt, D. R. and Smith, G. C. (2002): Prevalence and antibiotic susceptibility of *Salmonella* isolated from beef animal hides and carcasses. *Journal of Food Protection*, **65**, 284 - 290.

Bäumler, A. J., Tsoli, R. M., Ficht, T. A. and Adams L.G. (1998): Evolution of host adaptation in *Salmonella enterica. Infection and Immunity*, **66**, 4579–4587.

Boyd, D., Peters, G. A., Cloeckaert, A., Boumedine, K. S., Chaslus-Dancla, E., Imberechts, H. and Mulvey, M. R. (2001): Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. *Journal of Bacteriology*, **183**, 5725-5732.

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

Brown, M. and Eykyn, S. J. (2000): Non-typhoidal *Salmonella* bacteraemia without gastroenteritis: a marker of underlying immunosuppression. Review of cases at St. Thomas' Hospital 1970–1999. *Journal of Infection*, **41**, 256–9.

CDC (1991): Foodborne nosocomial outbreak of *Salmonella* Reading – Connecticut. *Morbidity and Mortality Weekly Report*, **40**, 804-806.

CDC (2000): Preliminary FoodNet data on the incidence of foodborne illnesses – selected sites, United States, 1999. *Morbidity and Mortality Weekly Report*, **49**, 201-205.

CDC (2003): Preliminary FoodNet data on the incidence of foodborne illnesses – selected sites, United States, 2002. *Morbidity and Mortality Weekly Report*, **52**, 340-343.

Chang, C-C., Lin Y-H., Chang, C-F, Yeh, K-S., Chiu, C-H., Chu C., Chien, M-S., Hsu Y-M, Tsai L-S. and Chiou C-S. (2005): Epidemiologic Relationship between fluoroquinolone-resistant *Salmonella enterica* serovar Choleraesuis strains isolated from humans and pigs in Taiwan (1997 to 2002). *Journal of Clinical Microbiology*, **43**, 2798–2804.

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

Clark, G. M., Kauffmann, A. F. and Gangerosa, E. J. (1973): Epidemiology of an international outbreak of *Salmonella* Agona. *Lance*t, **2**, 490-493.

Cody, S. H., Abbott, S. L., Marfin, A. A., Schulz, B., Wagner, P., K., Robbins, J., Mohle-Boetani, C. and Vugia, D. J. (1999): Two outbreaks of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw milk cheese, Northern California. *Journal of American Medical Association,* **281**,1805-1810.

Cogan, T.A. and Humphrey, T.J. (2003): The rise and fall of *Salmonella* Enteritidis in the UK. *Journal of Applied Microbiology*, **94**, 114S–119S.

Cooke, V. M., Miles, R. J., Price, R. G. and Richardson, A. C. (1999): A novel chromogenic ester agar medium for detection of salmonellae. *Applied and Environmental Microbiology*, **65**, 807–812.

Cormican, M, Butler, C., Morris, D., Corbett-Feeney, G. and Flynn, J. (1998): Antibiotic resistance amongst S. enterica species isolated in the Republic of Ireland. *Journal of Antimicrobial Chemotherapy,* **42**, 116-118.

D'Aoust, J-Y. (1989): *Salmonell*a. In: M.P. Doyle (ed.). Foodborne Bacterial Pathogens, Marcel Dekker Inc., New York, NY. pp. 327-412.

D'Aoust, J. -Y. (1997): *Salmonella* Species. In: Doyle, M. P., Beuchat, L. R., and Montville, T. J. (eds.): Food Microbiology- Fundamentals and Frontiers. ASM Press, Washington D. C. pp 129-158.

Daleel, E. E. and Frost, A. J. (1967): The isolation of *Salmonella* from cattle at Brisbane abattoir. *Australian Veterinary Journal,* **43**, 203-206.

Dargatz, D. A., Fedorka-Cray P. J., Ladely, C. A., Kopral, C. A., Ferris, K. E. and Headrick M.L. (2003): Prevalence and antimicrobial susceptibility of *Salmonella* spp. isolates from US cattle in feedlots in 1999 and 2000. *Journal of Applied Microbiology,* **95,** 753-761.

Davies, R. H. and Hinton, M. H. (2000): *Salmonella* in animal feed. In: Wray, C. and Wray, A. (eds): *Salmonella* in Domestic Animals. Walligford, Oxon, UK. CABI publishers. pp 285-300.

De Jong, H. and Ekdahl, M. O. (1965): Salmonellosis in calves-the effect of dose rate and other factors on transmission. *New Zealand Veterinary Journal,* **13**, 59-64.

Demissie, D. (2005): Prevalence and distribution of enterohemorrhagic *E. coli* O157:H7 and *Salmonella* serotypes isolated from selected samples in Debre Zeit and Addis Ababa, Ethiopia. Addis Ababa University, Faculty of Veterinary Medicine, DVM thesis

Doyle, M. P. and Cliver, D. O. (1990): *Salmonell*a. In: Cliver, D.O. (ed.): Foodborne Diseases. Academic Press, Inc. San Diego, California. pp 185-204.

Dunne, E. F., Fey P. D., Kludt, P., Reporter R., Mostasahri, F., Sillam, P., Wicklund, Miller. C. (2000): Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC beta lactamase. *Journal of the American Medical Association* **284**, 3151-3156.

Egan, J., Ward, J., Murphy, A. and Murray, G. (1999): Phage types of *Salmonella* Typhimurium isolated from cattle in the Republic of Ireland, 1992–1998. *Irish Veterinary Journal,* **52**, 325–331.

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

Evans, S. J. and Davies, R. H. (1996): Case control of multiple-resistant *Salmonella* Typhimurium DT 104 in cattle in Great Britain. *Veterinary Record*, **139**, 555-558.

Ewing, W. H. (1986): Serological Identification of *Salmonell*a. In: Ewing, W. H. (ed): Edwards and Ewing's Identification of *Enterobacteriacea*e. 4thed. New York: Elsevier science publishing Co., Inc. pp. 201-238.

FDA (1998): Bacteriological Analytical Manual, U.S. Food and Drug Administration, 8th ed. (rev. A). AOAC International, Gaithersburg, Md.

Fedorka-Cray, P. (1998): National Antimicrobial Resistance Monitoring System Enteric Bacteria (NARMS-EB): Veterinary Isolates. Final Report. www.ars-grin.gov

Fedorka-Cray, P. J., Kelly L. C., Stabel, T. J., Gay, J. T. and Laufer J. A. (1995): Alternate routes of invasion may affect pathogenesis of *Salmonella* Typhimurium in swine. *Infection and Immunity*, **63**, 2658-2664.

Fegan, N., Vanderlinde, P., Higgs, G. amd Desmarchelier, P. (2005): A study of the prevalence and enumeration of *Salmonella* enterica in cattle and on carcasses during processing. *Journal of Food Protection*, **68**, 1147-1153.

Fey, P. D., Safranek, T. J., Rupp, M. E., Dunne E. F., Ribot, E., Iwen P. C., *et al.* (2000): Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. *New England Journal of Medicin*e, **342**, 1242–9.

Fontaine, R. E., Arnon, S., Martin, W. T., Vernon, T. M., Jr, Gangarosa, E. J., Farmer, J. J., Moran, A. B., Silliker, J. H. and Decker, D. L. (1978): Raw hamburger: an interstate common source of human salmonellosis. *American Journal of Epidemiology*, **107,** 36–45.

Gedebou, M., and Tassew, A. (1981): Antimicrobial resistance and R factor of *Salmonella* isolates from Addis Ababa. *Ethiopian Medical Journal*, **19**, 77-83.

Germani, Y., Minssart, P., Vohito, M., Yassibanda, S., Glaziou, P., Hocquet, D., Berthe´Le´My, P. and Morvan, J. (1998): Etiologies of acute, persistent, and dysenteric diarrhoeas in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. *American Journal of Tropical Medicine and Hygiene*, **59**, 1008–1014.

Glickman, L. T., McDonough, P. L., Shin, S. J. and Fairbrother, J. M. (1981): Bovine salmonellosis attributed to *Salmonella* Anatum-contaminated haylage and dietary stress. *Journal of the American Veterinary Medical Association,* **178**, 1268-1272.

Glynn, M.K., Bopp, C., Dewitt, W., Dabney, P., Mokhtar, M. and Angulo, F.J. (1998): Emergence of multidrug-resistance *Salmonella enterica* serotype Typhimurium DT 104 infections in the United States. *New England Journal of Medicine,* **338**, 1333-1339.

Gomez, T. M., Motarjemi, Y., Miyagawa, S., Kaferstein, F. K. and Stohr, K. (1997): Foodborne salmonellosis. *World Health Statistics Quarterly,* **50**, 81–89.

Grau, F. H. and Brownlie, L. E. (1968): effect of some preslaughter treatments on the salmonella population in the bovine rumen and faeces. *Journal of Applied Bacteriology*, **31**,157-163.

Helms, M., Ethelberg S., Mølbak K. and the DT104 Study Group. (2005): International *Salmonella* Typhimurium DT104 infections, 1992–2001. *Emerging Infectious Diseases,* **11**, 859-867.

Hendriksen, M W., Orsel, K., Wagenaar, J. A., Miko, A. and van Duijkeren E. (2004): Animal-to-human transmission of *Salmonella* Typhimurium DT104A variant. *Emerging Infectious Diseases* **10**, 2225-1227.

Henson, S. (1997): Estimating the incidence of food-borne *Salmonella* and the effectiveness of alternative control measures using the Delphi method. *International Journal of Food Microbiology*, **35**, 195-204.

Heuzenroeder, M. W., Murray, C. J., Davos D. and Ross I.L (2004) *Salmonella* typing and colonisation of chickens by characterized *Salmonella* Sofia. Rural Industries Research and Development Corporation. RIRDC Publication No 04/138 RIRDC Project No IMV-3A.

Hollinger, K., Wray, C., Evans, S., Pascoe, S., Chapell, S. and Jones, Y. (1998): *Salmonella* Typhimurium DT104 in cattle in Great Britain. *Journal of the American Veterinary Medical Association,* **213**, 1732–1733.

Hunter, A. G., Linklater, K. A. and Scott J. A. (1976): Rodent vectors of *Salmonell*a. *Veterinary Record,* **99,** 145-146.

ISO (1998): Microbiology of food and Animal Feeding Stuff-Horizontal Method for the Detection of *Salmonell*a. ISO 6579, Geneva.

ISO (2002): Microbiology of food and Animal Feeding Stuff-Horizontal Method for the Detection of *Salmonell*a spp. ISO 6579, Geneva.

Jay, M. J. (2000): Modern Food Microbiology. 6th ed., Maryland: Aspen Publishers. pp 511-525.

Johnson, W. S., Mac Lachlan, G. K. and Hopkins, G. F. (1979): The possible involvement of seagulls *(Larus* spp.) in the transmission of *Salmonella* in dairy cattle. *Veterinary Record*, **105**, 526-527.

Judicial Commission of the International Committee on Systematics of Prokaryotes. (2005): The type species of the genus *Salmonella* Lignieres 1900 is *Salmonella enterica* (ex Kauffmann and Edwards 1952) Le Minor and Popoff 1987, with the type strain $LT2^{T}$, and conservation of the epithet *enterica* in *Salmonella enterica* over all earlier epithets that may be applied to this species. Opinion 80. *International Journal of Systematic and Evolutionary Microbiology*, **55**, 519–520.

Kopanic, R. J., Sheldon, B.W. and Wright, C. G. (1994). Cockroaches as vectors of S*almonell*a: laboratory and field trials. *Journal of Food Protection,* **57**, 124-132.

Krytenburg D. S, Hancock, D. D., Rice D. H., Besser, T. E., Gay C. C. and Gay, J. M. (1998): A pilot survey of *Salmonella enterica* contamination of cattle feeds in the Pacific northwestern USA. *Animal Feed Science and Technology* **75**, 75–79.

Lai, C-C., Lee L-N, Hsueh P-R., Yu C-J. and Yang P-C. (2005): Empyema thoracis from *Salmonella* Choleraesuis. *Emerging Infectious Diseases*, **11**, 1493-1494.

Levy, S. B. (1982): Microbial resistance to antibiotics: an evolving and persistent problem. *Lancet,* **10**, 83–88.

Mache, A. (2002): *Salmonella* serogroups and their antibiotics resistance patterns isolated from diarrhoeal stools of paediatric outpatient in Jimma Hospital and Jimma Health Center, South West Ethiopia. *Ethiopian Journal of Health Science*s, **12**, 37-46.

Mache, A., Mengistu, Y. and Cowley, S. (1997): *Salmonella* serogroups identified from adult diarrhoeal outpatients in Addis Ababa, Ethiopia: antibiotic resistance and plasmid profile analysis. *East African Medical Journal*, **74**, 183-186.

Maddox, C. W. (2003): *Salmonella* detection methods. In: Torrence, M. E., and Isaacson, R. E. eds.): Microbial Food Safety in Animal Agriculture- Current Topics. Iowa State Press, a Blackwell Publishing Company. pp 83-88.

MAFF (2000): *Salmonella* in livestock production. Veterinary Laboratories agency (Weybridge), Addlestone, UK, KT15 3NB.

Mateu, E. and Martin, M. (2001): Why is antimicrobial resistance a veterinary problem as well? *Journal of Veterinary Medicine Series B,* **48**, 569-581.

McCuddin, Z.P., Carlson, S.A.., Rasmussen, M.A. and Franklin, S.K. (2006*): Klebsiella* to *Salmonella* gene transfer within rumen protozoa: Implications for antibiotic resistance and rumen defaunation. *Veterinary Microbiology*, (in press).

McDonough, P. L., Fogelman, D., Shin, S. J., Brunner, M. A. and Lein, D. H. (1999): *Salmonella* enterica serotype Dublin infection: an emerging infectious disease for the Northeastern United States. *Journal of Clinical Microbiology*, **37**, 2418–2427.

McEvoy, J. M., Doherty, A. M., Sheridan, J. J., Blair, I. S. and McDowell D.A. (2003): The prevalence of *Salmonella* spp. in bovine faecal, rumen and carcass samples at a commercial abattoir. *Journal of Applied Microbiology*, **94**, 693–700

Mead, P. S., Slutsker, L., Dietz, V., McGaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M. and Tauxe, R. V. (1999): Food related illness and death in the United States. *Emerging Infectious Diseases,* **5,** 607–625.

Messele, G. and Alebachew, T. (1979): Antimicrobial susceptibility patterns and R-factor among *Salmonella* and *Shigella* isolates. *Ethiopian Medical Journal,* **18**, 7-14.

Millemann, Y., Gaubert, S., Remy, D. and Colmin C. (2000): Evaluation of IS200-PCR and comparison with other molecular markers to trace *Salmonella enterica* subsp. *enterica* serotype Typhimurium bovine isolates from farm to meat. *Journal of Clinical Microbiology,* **38**, 2204-2209.

Mølbak, K. (2004): Spread of resistant bacteria and resistance genes from animals to humans – the public health consequences. *Journal of Veterinary Medicine Series B,* **51**, 364-369.

Molla, B. and Mesfin, A. (2003): A survey of *Salmonella* contamination in chicken carcass and giblets in Central Ethiopia. *Revue de Médecine Véterinair*e, **154**, 267-270.

Molla, B., Berhanu, A., Muckle, A., Cole, L., Wilkie, E., Kleer, J. and Hildebrandt, G. (2006): Multidrug resistance and distribution of *Salmonella* serovars in slaughtered pigs. *Journal of Veterinary Medicine Series* B, **53**, 28-33.

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

Molla, B., Salah, W., Alemayehu, D. and Mohammed, A. (2004): Antimicrobial resistance pattern of *Salmonella* serotypes isolated from apparently healthy slaughtered camels *(Camelus dromedariu*s) in eastern Ethiopia. *Berliner und Münchener Tierärztliche Wochenschrift*, **117**, 39-45.

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

Molomo, M. A. (1998): A study on the prevalence and epidemiological key factors of *Salmonella* species in selected poultry farms in Debre Zeit, Ethiopia. Freie Universitaet Berlin and Addis Ababa University, MSc thesis.

Moo, D., O'Boyle, D., Mathers, W. and Frost A. J. (1980): The isolation of *Salmonella* from jejunal and caecal lymph nodes of slaughtered animals. *Australian Veterinary Journal*, **56**, 181-183.

Morgan, I. R., Krautil, F. L. and Craven, J.A. (1987): Effect of time in lairage on caecal and carcass *Salmonella* contamination of slaughter pigs. *Epidemiology and Infection*, **98**, 323-330.

Motsoela C., Collison, K. T. and Gashe, B. A. (2002): Prevalence of *Salmonella* in two Botswana abattoir environments. *Journal of Food Protection*, **65**, 1869 - 1872.

Murray, C. J. (1991): Salmonellae in the environment. *Review of Science and Technology Office Internationale des Epizooties,* **10**, 765-785.

National Committee for Clinical Laboratory Standards (1997): Performance standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from

animals and humans. Approved standard. NCCLS Document M31-A, NCCLS, Villanova, PA.

National Meteorological Service Agency (2003): Rainfall and temperature data, Addis Ababa, Ethiopia.

Nazer, A. H. K. and Osborne, A. D. (1976): *Salmonella* infection and contamination of veal calves: a slaughterhouse survey. *British Veterinary Journal,* **132**, 192-201.

Nyeleti, C., Molla, B., Hildebrandt, G. and Kleer, J. (2000): The prevalence and distribution of salmonellae in slaughter cattle, slaughterhouse personnel and minced beef in Addis Ababa, Ethiopia. *Bulletin of Animal Health and Production in Afric*a, **48**, 19-24.

O'Brien, T .F. (2002): Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clinical Microbiology and Infectious Diseases,* **34** (Suppl.3), S78-S84.

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

Patterson, S. and Isaacson, R. E. (2003): Genetics and pathogenesis of *Salmonell*a. In: Torrence, M. E., and Isaacson, R. E. (eds.): Microbial food Safety in Animal Agriculture-Current Topics. Iowa State Press, a Blackwell Publishing Company. pp 89-96.

Pegram, R. G., Roeder, P. L., Hall, M. L. M. and Rowe, B. (1981): *Salmonella* in livestock and animal by-products in Ethiopia. *Tropical Animal Health and Productio*n, **13**, 203-207.

Perry, J., M., Taylor, J., Amanda L., Freeman R. and Gould F. K. (1999): ABC Medium, a new chromogenic agar for selective isolation of *Salmonella* spp. *Journal of Clinical Microbiology,* **37**, 766–768

Piddock, L. J. (2002): Fluoroquinolone resistance in *Salmonella* serovars isolated from humans and food animals. *FEMS Microbiology Reviews,* **26**, 3–16.

Popoff, M. Y., and Le Minor, L. (1997): Taxonomy of the genus *Salmonell*a: changes in serotypes nomenclature. In: Antigenic formulas of the *Salmonella* serotypes, 7th revision. WHO Collaborating Center for Reference and Research on *Salmonell*a. Pasteur Institute, Paris, France.

Poppe, C., Ziebell, K., Markey, B. and Allen, K. (2002): Diversity in antimicrobial resistance and other characteristics among *Salmonella* Typhimurium DT 104 isolates. *Microbial Drug Resistance*, **8**, 107-121.

Puyalto, C., Colmin, C. and Laval, A. (1997): *Salmonella* Typhimurium contamination from farm to meat in adult cattle: descriptive study. *Veterinary Researc*h, **28**, 449-460.

Quinn, P. J., Carter, M. E., Markey, B., and Carter, G.R. (1999): *Enterobacteriacea*e. In: Clinical Veterinary Microbiology. Molsby International Limited. pp 226-234.

Rabsch, W., Altier, C., Tschape, H. and Baumler, A. J. (2003): Foodborne *Salmonella* infections. In: Torrence, M. E. and Isaacson, R. E. (Eds.): Microbial Food Safety in Animal Agriculture: Current Topics. Iowa State Press. A Blackwell publishing company. pp 97- 107.

Radostits, O. M., Blood, D. C. and Gay, C. C. (1994): Veterinary Medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. 8th ed. London: Bailliere Tindall. pp 730-745.

Rambach, A. (1990): New plate medium for facilitated differentiation of *Salmonella* spp. from *Proteus* spp. and other enteric bacteria. *Applied and Environmental Microbiology* **56**, 301–303.

Rankin, S. C., Aceto, H., Cassidy, J., Young, S., Love, B., Tewari, D. and Munro, D. S. (2002): Molecular characterization of cephalosporin-resistant *Salmonella enterica* serotype Newport isolates from animals in Pennsylvania. *Journal of Clinical Microbiology*, **40**, 4679-4684.

Reid, C.-A., Small, A., Avery, S. M. and Buncic. S. (2002): Presence of foodborne pathogens on cattle hides. *Food Control*, **13**, 411-415.

Richardson, A. (1975): Salmonellosis in cattle. *Veterinary Record*, **96**, 329-331.

Rodrigues, D.C., Tauxe, R. V. and Rowe B. (1990): International increase in *Salmonella* Enteritidis: a new pandemic? *Epidemiology and Infection*, **105**, 21–27.

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

Schlosser, W., Hogue, A., Ebel, E., Rose, B. Umholtz, R., Ferris, K. and James, W. (2000): Analysis of *Salmonella* serotypes from selected carcasses and raw ground products sampled prior to implementation of the pathogen reduction; Hazard Analysis and Critical Control point Final Rule in the U.S. *International Journal of Food Microbiology*, **58**, 107-111.

Schroeter, A., Hoog, B. and Helmuth, R. (2004): Resistance of *Salmonella* Isolates in Germany. *Journal of Veterinary Medicine Series B*, **51**, 389-392

Shipp, C. R. and Rowe, B. (1980): A mechanised microtechnique for *Salmonella* serotyping. *Journal of Clinical Pathology*, **33**, 595-597.

Smeltzer, T., Thomas, R. and Collins, G. (1980a): The role of equipment having accidental or indirect contact with the carcass in the spread of *Salmonella* in an abattoir. *Australian Veterinary Journal*, **56**, 14-17.

Smeltzer, T., Thomas, R. and Collins, G. (1980b): Salmonellae on posts handrails and hands in beef abattoir. *Australian Veterinary Journal*, **56**, 184-186.

Smerdon W. J., Adak, G. K., O'Brien, S. J., Gillespie, I. A. and Reacher, M. (2001): General outbreaks of infectious intestinal disease linked with red meat, England and Wales, 1992-1999. *Communicable Diseases and Public Health,* **4**, 259-267.

Stata Corporation (2001): Stata Stastical Software. Release 7.0. College Station, Texas.

Stolle A. (1981): Spread of salmonellas during cattle slaughtering. *Journal of Applied Bacteriology,* **50**, 239-245.

Su, L-H., Chiu, C-H., Chu,C., Wang, M-H., Chia, J-H, and Wu, T-L. (2003): In vivo acquisition of ceftriaxone resistance in *Salmonella enterica* serotype Anatum. *Antimicrobial Agents and Chemotherapy*, **47**,563–567.

Sumner J., Raven, G. and Givney R. (2004): Have changes to meat and poultry food safety regulation in Australia affected the prevalence of *Salmonella* or of salmonellosis? *International Journal of Food Microbiology*, **92**, 199– 205.

Swartz, M. N. (2002): Human diseases caused by foodborne pathogens of animal origin. *Clinical Microbiology and Infectious Dis*eases, **34**, (Suppl 3), S111–22.

Tablante, N. L. and Lane, V. M. (1989): Wild mice as potential reservoirs of *Salmonella* Dublin in a closed dairy herd. *Canadian Veterinary Journal*, **30**, 590-592.

Tauxe, R. (1999): *Salmonella* Enteritis and *Salmonella* Typhimurium DT104. Successful subtypes in the modern world. In: Emerging Infections. Washington: ASM Press. pp. 37–52.

Tauxe, V. T. (1991): *Salmonella*; A postmodern pathogen. *Journal of Food Protection,* **54**, 563-568.

Thamlikitkul V., Dhiraputra C., Paisarnsinsup T. and Chareandee C. (1996): Non-typhoidal *Salmonella* bacteraemia: clinical features and risk factors. *Tropical Medicine and International Health*, **1**, 443–448.

Threlfall, E. J., Ward, L. R. and Rowe, B. (1997): Increasing incidence of resistance to trimethoprim and ciprofloxacin in epidemic *Salmonella* Typhimurium DT104 in England and Wales. *Eurosurveillance Report*, **2**, 81–84.

Thrusfield, M. (1995). Veterinary Epidemiology. 2nd ed. Oxford: Blackwell Science Ltd. pp. 182-198.

Tibaijuka, B., Molla, B., Hildebrandt, and G., Kleer, J. (2003): Occurrence of salmonellae in retail raw chicken products in Ethiopia. *Berliner und Münchener Tierärztliche Wochenschrift*, **116**, 55-58.

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 Afric*a, **50**, 86-95.

Tizzard, I. R., Fisk, N. A. and Harmeron, J. (1979): Free flying sparrows as carriers of salmonellosis. *Canadian Veterinary Journal*, **20**, 143-144.

Urfer, E., Rossier, P., Me, F., Krending, M-J., Burnens, A., Bille J., Francioli, P. and Zwahlen, A. (2000): Outbreak of *Salmonella* Braenderup gastroenteritis due to contaminated meat pies: clinical and molecular epidemiology. *Clinical Microbiology and Infection*, **6**, 536-542.

van Duijkeren, E., Wannet, W. J. B., Houwers, D. J. and van Pelt, W. (2002): Serotype and phage type distribution of *Salmonella* strains isolated from humans, cattle, pigs, and chickens in The Netherlands from 1984 to 2001. *Journal of Clinical Microbiology*, **40**, 3980-3985.

Venter, B.J., Myburgh, J. G., and Van der Walt, M. L. (1994): Bovine salmonellosis In: Coetzer J.W., Thomson, G.R., and Tustin, R. C (eds). Infectious Diseases of

Livestock.with special reference to Southern Africa. Oxford University Press. Cape town. pp 1104-1112.

Waltman, W. D. and Mallinson, E. T. (1995): Isolation of *Salmonella* from tissue and environmental samples: a nationwide survey. *Avian Diseases,* **39**, 45-54.

Watson, A. (1975): Salmonellosis and meat hygiene: red meat. *Veterinary Record,* **96**, 374 - 376.

WHO (1998): The Medical Impact of the Use of Antimicrobials in Food Animals: Report of the WHO Meeting, Berlin, Germany; 13-17 October 1997. www.aphis.usda.gov.

WHO. (2001): WHO Global Strategy for Containment of Antimicrobial Resistance. WHO, Geneva, Switzerland. pp. 1-92.

Winokur, P.L., Vonstein, D. L., Hoffman, L. J., Uhlenhopp, E. K. and Doern, G. V. (2001): Evidence for transfer of CMY-2 AmpC beta lactamase plasmids between *Eschericia coli* and *Salmonella* isolates from food animals and humans. *Antimicrobial Agents and Chemotherapy*, **45**, 2716-2722.

Witte, W. (1998): Medical consequences of antibiotic use in agriculture. *Science*, **279**, 996-997.

Woldemariam, E., Molla, B., Alemayehu, D. and Muckle, A. (2005): Prevalence and distribution of *Salmonella* in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. *Small Ruminant Research*, **58**, 19-24.

Wray, C. (1991): Salmonellosis in cattle. *In Practice,* **13**,13-15.

Wray, C. and Sojka, W. J. (1977): Reviews of the progress of dairy science: bovine salmonellosis. *Journal of Dairy Research,* **44,** 383-425.

Wray, C. and Davies, R. H. (2000): *Salmonella* infection in cattle. In:Wray, C. and Wray A (eds.). *Salmonella* in Domestic Animals. New York, CABI publishing. pp 169-190.

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. Iowa State Press, a Blackwell Publishing Company. pp 73-82.

Wright, J. G., Leslie, A. T., Smith, K. E., Bender, J. B., Frank, R. K., Grendon, J. H., Rice, D. H., Thiessen, A. B., Gilbertson, C. J., Sivapalasingam, S., Barrett, T. J., Besser, T. E., Hancock, D. D. and Angulo, F. J. (2005): Multidrug-resistant *Salmonella* Typhimurium in four animal facilities. *Emerging Infectious Diseases*, **11**, 1235-1241.

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

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

8. APPENDICES

Appendix 1: Media used and preparations for the isolation and identification of *Salmonella*

1. Buffered peptone water (BPW) (AES loboratoire, Cedex, France)

Composition (g/liter): Peptone from casein 10.0; sodium chloride 5.0; di-sodium hydrogen phosphate 3.5; potassium dihydrogen phosphate1.5 *Preparation:* Twenty grams of this medium was dissolved in one liter of distilled water and sterilized by autoclaving at 121^{o}C for 15 minutes.

2. Rappaport-Vassiliadis (RV-Medium) (Difco™, Becton Dickinson, USA)

Composition (g/liter): Pancreatic digest of casein 4.54; Sodium chloride 7.2; Monopotassium phosphate 1.45; Magnesium chloride (anhydrous) 13.4; Malachite green oxalate 0.036.

Preparation: Suspend 26.6 grams of the powder in 1 liter of purified water. Mix thoroughly. Warm slightly to completely dissolve the powder. Dispense 10 ml amounts into suitable containers, autoclave at 116^{o}C for 15 minutes. Test samples of the finished product for performance, using stable, typical control cultures.

3. Selenite Cystine broth (Difco, Becton Dickinson, Claix, France**)**

Composition (g/liter): Pancreatic digest of casein 5.0; lactose 4.0; sodium selenite 4.0; sodium phosphate 10.0

Preparation: Suspend 23 grams of the powder in 1 liter of distilled water. Heat to boiling. Avoid overheating. Do not autoclave.

4. Brilliant green-phenol-red agar (BPLS Agar) (Merck, Darmstadt, Germany)

Composition (g/liter): Peptone 10.0; meat extract 5.0; yeast extract 3.0; lactose 10.0; saccharose 10.0; disodium hydrogen phosphate 1.0; sodium dihydrogen phosphate 0.6; brilliant green 0.005; phenol red 0.09; agar 10.0

Preparation: Forty-nine point seven (49.7) grams of the powder was suspended in one liter of distilled water, carefully brought to the boil with frequent agitation to dissolve completely, mixed well and poured into petridishes.

5. Xylose lysine desoxycholate agar (XLD-agar) (AES laboratoire, Cedex, France)

Composition (g/liter): Yeast extract 3.0; L-Lysine hydrochloride 5.0; xylose 3.75; lactose 7.5; sucrose 7.5; sodium desoxycholate 1.0; sodium chloride 5.0; sodium thiosulphate 6.8; iron (iii) ammonium citrate 0.8; phenol red 0.08; agar 16.5

Preparation: Fifty-seven grams of the powder was suspended in one liter of distilled water, brought to the boil with frequent agitation to dissolve completely, mixed well and poured into Petri dishes.

6. Triple sugar iron agar (TSI) (Difco, Becton Dickinson, Claix, France)

Composition (g/liter): Beef extract 3.0; yeast extract 3.0; pancreatic digest of casein 15.0; proteose peptone No.3 5.0; dextrose 1.0; lactose 10.0; sucrose 10.0; Ferrous sulfate 0.2; sodium chloride; 5.0; Sodium thiosulfate 0.3; Agar 12; Phenol red 0.024.

Preparation: Suspend 65 grams of the powder in 1 liter of purified water, mix thoroughly. Heat with frequent agitation and boil for 1minute to completely dissolve the powder. Dispense into tubes and autoclave at 121^{o}C for 15 minutes. Cool in a slanted position so that deep butts are formed.

7. Lysine iron agar (LIA) (Difco^{TM}, Becton Dickinson, Claix, France)

Composition (g/liter): Peptone 5.0; yeast extract 3.0; dextrose 1.0; L-Lysine HCl 10.0; Ferric ammonium citrate 0.5; Sodium thiosulphate 0.04; bromcresol purple 0.02; Agar 15.0.

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 to completely dissolve the powder. Autoclave at 121^{o}C for 12 minutes.

8. Urea agar base (BBL$^{®}$, Becton Dickinson, USA)

Composition (g/liter): Pancreatic Digest of Gelatin 1.0; dextrose 1.0; sodium chloride 5.0; potassium phosphate 2.0; Urea 20; Phenol red 0.012

Preparation: Suspend 29g of the powder in 100 ml of distilled water. Mix thoroughly and sterilize by filtration. Suspend 15g of Agar in 900 ml distilled water. Autoclave at 121^{o}C for 15 minutes. Cool to 50^{o}C and add 100 ml of filter sterilized urea agar base. Mix thoroughly and dispense aseptically in sterile tubes. Cool tubed medium in a slanted position so that deep butts are formed.

9. Simmons Citrate Agar (Difco, Detroit, USA**)**

Composition (gram/liter): Magnisium sulphate 0.2g, amonium dihydrogen sulphate 1.0, dipotassium phosphate 1.0, sodium citrate 2.0, sodium chloride 5.0, Bacto agar 15.0, Bacto bromthymol blue 0.08

*Preparatio*n: Suspend 24.2g in 1 liter of distiled or deionized water and boil to disolve completely. Sterilization at 121-124oc for 15 minutes

10. Rambach® agar (Merck, Darmstadt, Germany)

Composition (g/liter): Peptone 8.0; sodium chloride 5.0; sodium deoxycholate 1.0; chromogenic mix 1.5; propylene glycol 10.5; agar-agar 15.0

Preparation:

i. One vial of liquid mix was added to 250 or 1000 ml distilled water and mixed by swirling until completely dissolved (The water quantity is dependent on the respective pack size.)

ii. One vial of nutrient-powder was added and mixed by swirling until completely suspended.

iii. The medium was heated in a boiling water bath until totally dissolved, while carefully shaking from time to time.

iv. The medium was cooled as fast as possible in a water-bath (45-50^{o}C). During this procedure (max. 30 minutes) it gently shook from time to time and poured in to plates.

v. In order to prevent any precipitate or clotting of the chromogenic-mix in the plates, petri dishes were placed on a cool surface during pouring procedure.

11. Tryptic soy agar (Difco, Sparks, USA)

Composition (g/liter): Pancreatic digest of casein 15.0; enzymatic digest of soybean meal 5.0; sodium chloride 5.0; agar 15.0

Preparation: forty grams of the powder was suspended in 1 litre of distilled water and mixed thoroughly. The medium was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder. Then it was autoclaved at 121^{o}C for 15minutes, dispensed into transporting tubes and allowed the medium to solidify.

12. Brain heart infusion agar (Difco, Becton Dickinson, Claix, France)

Composition (g/liter): Infusion from calf brains 200.0, infusion from beef heart 250.0, proteose peptone 10.0, dextrose 2.0, sodium chloride 5.0, disodium phosphate 2.5, agar 14.0.

Preparation: Suspend 52 grams in 1 liter distilled water and heat to boiling to dissolve completely. Sterilize in the autoclave for 15 minutes at 121^{o}C.

13. Muller-Hinton Agar (BBL®, Becton Dickinson, USA)

Composition (g/liter): Meat infusion 5.0; casein hydrolysate 17.5; starch 1.5; agar-agar 13.

Preparation: Dissolve 34.0 g in 1 litre of distilled water, boil to homogenize it, autoclave at 115°C for 10 min, cool to 50°C and dispense on to sterile petridishes

Appendix 2: Preparation of 0.5 McFarland Turbidity Standard.

Solution A (0.048 M $BaCl_2$):

1.175 g $BaCl_2.2H_2O$

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 100 ml with distilled water

For standard:

Add 0.5 ml solution A to 95.5 ml of solution B.

Shake vigorously and dispense into 4-5ml sealed screw capped vials. Store in the dark at room temperature.

Appendix 3: Work sheet for recording colony morphology and biochemical reactions of presumptive *Salmonella* isolates

Sample No.	Date of Collection	Sample type	Origin	sex	Media		Colony characters	Isolate No.	TSI				LIA			Urease	Cittrate
					enrichment	plating			Lactose/ sucrose	Dextrose	H2S	gas	H2S	lysine decarbo-xylase	lysine deam-inase		

9. CURRICULUM VITAE

A. Biographical Data:

Name	Berhanu Sibhat
Date of birth	December 8, 1972.
Place of birth	Ejersa Goro, East Hararghe, Ethiopia
Marital status	Married
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Senior instructor at Alage Agricultural Technical and Vocational Training Collage

B. Educational background

Year	School Attended
1979- 1986	Jarsso Elementary and junior school, Ejersa Goro
1986 -1991	Harar Senior secondary school, Harar
1991 -1997	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia

C. Work Experience

November 2000 to August 2004	Government employed field veterinarian in three Woredas of Bale zone of the Oromia National Regional State
Since July 2004 to date	Senior instructor at Alage Agricultural Technical and Vocational Training Collage, Head of Animal Health Department the last two years prior to joining the graduate program.

D. Research output/Technical paper

Prevalence of bovine mastitis in dairy farms in and around Dire Dawa Administrative Council and Eastern Hararghe Zone (DVM Thesis, presented to AAU, FVM, 1997).

E. Membership to professional societies

Member of Ethiopian Veterinary Association.

F. Language

Amharic	Writing and speaking
Afan Oromo	Writing and speaking
English	Writing and speaking

G. Computer Skill

MS Dos, MS Word, MS Excel

10. SIGNED DECLARATION SHEET

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

Name _____

Signature _____

Date of submission _____

This thesis has been submitted for examination with our approval as University advisor.

Dr. Bayleyegn Molla (DVM, MSc, PhD. Assoc. Prof.) _____

Dr. Ademe Zerihun (DVM, MSc, PhD, Ass. Prof.) _____