ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

SALMONELLA IN APPARENTLY HEALTHY SLAUGHTERED SWINE IN ADDIS ABABA, ETHIOPIA

By

KASSAYE ARAGAW LIDETE

JUNE 2005

DEBRE ZEIT, ETHIOPIA

1094

ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

SALMONELLA IN APPARENTLY HEALTHY SLAUGHTERED SWINE IN ADDIS ABABA, ETHIOPIA

A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Medicine

By

KASSAYE ARAGAW LIDETE

JUNE 2005

DEBRE ZEIT, ETHIOPIA

SALMONELLA IN APPARENTLY HEALTHY SLAUGHTERED SWINE IN ADDIS ABABA, ETHIOPIA

By

KASSAYE ARAGAW LIDETE

Board of Examiners:	Signature
1. Prof. Ph. Dorchies	
2. Dr. David Barrett	
3. Dr. Andy Catley	
4. Dr. Mohammed Abdella	

Academic Advisor:

Dr. Bayleyegn Molla

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES AND APPENDICES

LIST OF ABBREVIATIONS

AAU	Addis Ababa University
BG	Brilliant green agar
BPW	Buffered peptone water
CC	Cecal content
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CS	Carcass swab
Defra	Department for Environment, Food and Rural Affairs
DT	Definitive type
DVM	Doctor of Veterinary Medicine
FDA	Food and Drug Administration
FVM	Faculty of Veterinary Medicine
H	Flagella antigen
HE	Hektoen enteric agar
H_2S	Hydrogen Sulphide
ISO	International Organization for Standardization
LIA	Lysine iron agar
LPS	Lipopolysaccharide
LT	Labile toxin
MLN	Mesenteric lymph nodes
MSc	Master of Science
NCCLS	National Committee for Clinical Laboratory Standards
O	Somatic antigen
°C	Degree celsius
OIÉ	Office International des Épizooties
OR	Odds ratio
RV	Rappaport- Vassilliadis broth
ST	Stable toxin
TSB	Trypticase soy broth

TSI	Triple sugar iron agar
TT	Tetrathionate
USA	United States of America
Var.	variety
Vi	Capsular antigen
WHO	World Health Organization
XLD	Xylose lysine desoxycholate agar

ACKNOWLEDGEMENTS

I would like to express my deep sense of indebtedness to Dr. Bayleyegn Molla, my academic advisor, for his scholarly guidance, unreserved reference material provision and encouragement during the research work. His skillful, meticulous, correction of the manuscript also deserves appreciation.

The Amhara Regional Agricultural Research Institute (ARARI) and Sheno Agricultural Research Center and their respective officials deserve special thanks for allowing me to join the graduate school at the last minute.

I wish to express my sincere thanks to members of meat inspection team at Addis Ababa abattoir, especially Ato Negussie Mamuye, and the abattoir technical personnel, for their help and cooperation during sample collection.

I am most grateful to Dr. Anne Muckle, head of the Laboratory for Foodborne Zoonoses, Health Canada, Dr. Cornelius Poppe and other technical staff at the OIÉ Reference Laboratory for Salmonellosis, Health Canada, Canada for serotyping, phage typing and antimicrobial resistance testing of our *Salmonella* isolates.

A lot of thanks are due to friends, Assefa W., Desalegne M., Messele A. and extern students Ayalew, Dagnachew and Teklesselassie who made the at times difficult situation in the lab pleasant through humors and understanding.

Staffs of the microbiology laboratory at FVM, especially Tsehay deserve special appreciation for their support, understanding and tolerance during the height of our work in the laboraroty.

Last but not least I sincerely acknowledge my parents Aragaw and Tiruwork, my wife Amsalegenet W/Senbet and my sisters and brothers: Fantu, Negatu, Yosef, Genet and Aschalew for their love and support throughout my study.

ABSTRACT

A cross-sectional study was conducted on apparently healthy slaughtered swine at Addis Ababa abattoir from October 2004 to February 2005. The objectives of the study were to estimate the prevalence of *Salmonella* in slaughter swine, to find out *Salmonella* serovars distribution, to determine the antimicrobial resistance of *Salmonella* isolates to selected antimicrobial agents and to investigate the association between some potential risk factors and *Salmonella* contamination of carcasses. A total of 849 samples, consisting of cecal content (n = 278), mesenteric lymph nodes (n = 278), carcass swab (n = 277) and scalding water (n = 16) samples were collected. 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 849 samples, 173 (20.4%) were *Salmonella* positive. Sixty-three of 278 (22.7%) cecal content, 99 of 278 (35.6%) mesenteric lymph nodes and 11 of 277 (4.0%) carcass swab samples were found positive for *Salmonella*. By considering an animal positive for *Salmonella* when it is positive either for cecal content or mesenteric lymph nodes or for both, 120 (43.2 %) swine were found infected with *Salmonella*. None of the scalding water samples were positive.

There was a statistically significant association between cecal content *Salmonella* status and carcass contamination with *Salmonella* at individual level. Swine that carry *Salmonella* in their cecal content were 6.7 (OR = 6.7, 95% CI = [1.90-23.76]) times more likely to end up as contaminated carcasses than free animals.

Among 173 *Salmonella* isolates 166 were identified as specific serovars and grouped into 17 serotypes. *Salmonella* Hadar (27.2%), *S.* Eastbourne (22.5%) and *S.* Saintpaul (21.4%) were the most prevalent serovars accounting for 71.1% of total isolates. Other serotypes isolated include *S.* Typhimurium (5.2%), *S.* Typhimurium var. Copenhagen (3.5%), *S.* Kentucky (2.9%) *S.* Enteritidis (2.3%) and *S.* Newport (2.3%). Two serotypes, *S.* Amager and *S.* Tarshyne were reported for the first time in Ethiopia. Serotypes predominantly isolated in this study were different from serovars frequently reported from swine in other countries. Among serotypes

which had more than 5 isolates; *S.* Hadar and *S.* Eastbourne were isolated from all sample types, while *S.* Saintpaul and *S.* Typhimurium var. Copenhagen were isolated from CC and MLN. *Salmonella* Typhimurium was isolated only from MLN.

All isolates were tested for susceptibility to a panel of 24 selected antimicrobials. Out of the 173 isolates tested, 57 (32.9%) were resistant. All the resistant isolates were multidrug resistant from two to up to ten different antimicrobials. Resistance was associated with serotype. Among the 17 serotypes identified, resistant isolates belonged to only 4: *S.* Hadar, *S.* Kentucky, *S.* Typhimurium and *S.* Braenderup. Most frequent resistance was encountered for streptomycin (32.4%), followed by tetracycline (31.8%) and nitrofurantoin (27.2%). The most common pattern of resistance observed was to nitrofurantoin, streptomycin and tetracycline representing 82.5% of resistant isolates. *Salmonella* Kentucky, *S.* Typhimurium and *S.* Braenderup were resistant to 4 up to 10 different antimicrobials.

Results of this study showed the potential risk *Salmonella* from swine pose to public health in Ethiopia as sources of pathogenic and multi-drug resistant serovars. The risk factors associated with high prevalence of *Salmonella* in swine need to be identified, so that appropriate control measures could be implemented. It is also necessary to determine the role of the serovars identified, in salmonellosis, both in swine and human beings in Ethiopia.

Key words: Swine, *Salmonella*, serotypes, prevalence, antimicrobial resistance, Addis Ababa, 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 serovars 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. All strains of *Salmonella* are considered pathogenic to humans (Doyle and Cliver, 1990; Radostits *et al.*, 1994)

Salmonellosis is an important zoonotic infection, perhaps the most widespread zoonosis in the world, and human salmonellosis causes wide spread morbidity and economic loss (Acha and Szyfres, 2001; Wray and Davies, 2003). Salmonellosis is the leading cause of foodborne illness in many countries in the world. It is more widespread in young children, in elderly citizens frequently affected with underlying chronic diseases, and immuno-suppressed individuals. Human salmonellosis from the consumption of contaminated foods generally remains on the increase world wide causing pain, suffering and loss of leisure time (Radostits *et al.*, 1994; D'Aoust, 1997).

Salmonellosis is a significant cause of economic loss in farm animals because of the cost of clinical disease, which include death, abortion, diagnosis, and treatment of clinical cases, diagnostic laboratory costs, and the cost of control and prevention. The losses incurred by the livestock producers include reduced feed efficiency, and reduced weight gains and milk production in dairy herds because of salmonellosis (D'Aoust, 1989; Radostits *et al.*, 1994).

Salmonellae are a major cause of disease in swine and have a significant economic impact. For instance, it used to cost US producers 100 million USD per year nationally 12 years ago (Roof *et al.*, 1993). Clinical disease is usually caused by *Salmonella* Choleraesuis. Occasionally, *S.* Typhimurium or, rarely, *Salmonella* Typhisuis is involved in localized epizootics. Infection with other serotypes rarely causes disease unless a naïve or compromised host ingests numerous

organisms or a carrier becomes immunocompromised. Salmonellosis as a disease in swine is reported worldwide and occurs mainly in weaned swine. Increased incidence of the disease tends to be present in geographic areas of intensive swine production, particularly in areas in which swine are transported and commingled (Schwartz, 1993).

Most *Salmonella* are not host-adapted and are considered potential pathogens for humans. In swine, infection with a variety of serotypes is common; few serotypes are significant causes of disease. *Salmonella* infections in swine are important because of the zoonotic potential of infected pork products and the effects of the actual disease in swine (Schwartz, 1993).

The economic losses associated with human salmonellosis are associated with investigation, treatment, and prevention of illness. It may also involve productivity losses and absenteeism, costs of food safety regulatory programs and costs to the food industry for product recalls, negative publicity and plant closures due to foodborne salmonellosis outbreaks (D'Aoust, 1989; Radostits *et al.*, 1994; Wray and Davies, 2003).

Salmonella Typhimurium was the most frequently isolated cause world wide of *Salmonella* gastroenteritis, bacteremia, and asymptomatic carriage. However, since early nineties *S.* Enteritidis has been the most frequently isolated serovar. Other common human serotypes are *S.* Newport, *S.* Infantis, and *S.* Heidelberg, their relative frequency varying with time and geographic localities (Davis *et al.*, 1990; van Duijkeren *et al.* 2002). Serotypes adapted to a particular animal species are usually less pathogenic for man (*S.* Pullorum, *S.* Gallinarum, *S.* Abortusequi, *S.* Abortusovis). An exception is *S.* Choleraesuis, which produces a serious disease (Acha and Szyfres, 2001).

A high proportion of *Salmonella* strains with multiple antibiotic resistance has been observed in many countries. The main causes of this in industrialized countries has been the overuse and misuse of antibiotics in animal feed as a growth enhancer, as well as the indiscriminant prescription-drug treatment of people and animals. In developing countries, the principal cause of the emergency of multiresistant *Salmonella* strains may be self-medication, made possible by the public's easy access to antibiotics without a prescription and indiscriminant and continuous use of subtherapeutic doses of antimicrobials, without any prescription in animals (Acha and

Szyfres, 2001). High level of antimicrobial resistance was recorded in Ethiopia on isolates from animals and animal products (Tibaijuka *et al.*, 2002; Alemayehu *et al.*, 2003; Molla *et al.*, 2003b; Molla *et al.*, 2004b; Molla, 2004; Zewdu, 2004) and human beings (Mache *et al.*, 1997; Mache, 2002).

Foods containing products from farm animals, especially from poultry, swine, and cattle, are important sources of human *Salmonella* infections (van Duijkeren *et al.*, 2002). Reports indicate the occurrence of *Salmonella* in livestock and livestock products, including swine and pork, in Ethiopia (Pegram *et al.*, 1981; Molomo, 1998; Nyeleti *et al.*, 2002; Tibaijuka *et al.*, 2002; Alemayehu *et al.*, 2003; Woldemariam *et al.*, 2005; Molla and Mesfin, 2003; Ejeta *et al.*, 2004; Molla *et al.*, 2004a; Molla, 2004; Zewdu, 2004). Nontyphoid *Salmonella* were also identified as important causes of diarrhoea in Ethiopia (Mache, 2002; Ashenafi and Gedebou, 1985; Mache *et al.*, 1997). Surveillance of *Salmonella* serovars and phage types from human and animal sources is relevant for detecting outbreaks, for identifying sources of infection and for implementing prevention and control measures (van Duijkeren *et al.*, 2002). Therefore this study was conducted with the objectives to:

- Determine the prevalence of *Salmonella* in swine at slaughter.
- Identify serotypes and phagetypes prevalent in swine.
- Establish antimicrobial susceptibility/resistance patterns of *Salmonella* isolates from swine and
- Demonstrate carcass contamination level and show some of the risk factors associated with carcass contamination in swine in the abattoir.

2. LITERATURE REVIEW

Salmonellae are small, Gram-negative, non-sporing, facultative anaerobic, rod-shaped, motile bacteria that belong 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 metabolize nutrients by the respiratory and fermentative pathways (D'Aoust, 1997). The genus *Salmonella* obtained its name from the American veterinarian Daniel E. Salmon, who first isolated *S. enterica* serotype Choleraesuis from swine in 1885 (Rabsch *et al.*, 2003). These bacteria can resist dehydration for a very long time, both in feces and in foods for human and animal consumption (Acha and Szyfres, 2001).

According to Jay (2000) for epidemiological purposes, the salmonellae can be placed into three groups:

- Those that infect humans only: These include *S.* Typhi, *S.* Paratyphi A and *S.* Paratyphi C. This group includes the agents of typhoid and the paratyphoid fevers, which are the most severe of all diseases caused by salmonellae.
- The host adapted serovars (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 serovars (no host preference). These are pathogenic for humans and other animals, and they include most foodborne serovars.

On the other hand Uzzau *et al.* (2000) proposed adoption of the following terms to describe *Salmonella* host-adaptation, to avoid confusion and contradiction within the literature: *Salmonella* serotypes which are almost exclusively associated with one particular host species to be referred as host-restricted serotypes. Serotypes which are prevalent in one particular host species but which can also cause disease in other host species to be referred as host-adapted serotypes and ubiquitous serotypes to be referred as un-restricted serotypes.

2.1. Taxonomy and nomenclature

Salmonella nomenclature is complex, and scientists use different systems to refer to and communicate about this genus. It is still evolving and the debate on the name for the type species is not likely to be settled any time soon (Brenner *et al.*, 2000). Various classification schemes have been proposed, leading to controversy and confusion. At present, the scheme conceived by Kauffmann-White is used. The Kauffmann-White scheme divides salmonellae into serotypes. O (somatic), H (flagellar), and Vi (capsular) antigens are distinguished primarily on the basis of their antigenic structure (Acha and Szyfres, 2001). Many workers accept a scheme proposed by Le Minor and Poppof (1987) of the WHO collaborative center for reference and research on *Salmonella* including CDC (Brenner *et al.*, 2000).

According to the scheme proposed by Le Minor and Poppof (1987), the genus *Salmonella* consists of two species (1) *S. enterica* which is divided into six sub species: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. entericae* subsp. *diarizonae*, *S. entericae* subsp.*houtenae* and *S. enterica* subsp. *indica*; and (2) *S. bongori* (formerly called *S. enterica* subsp. *bongori*) (Popoff and Le Minor, 1997).

Serotype names designated by antigenic formulae include the following:

I) Subspecies designation (subspecies I through VI).

II) O (somatic) antigens followed by a colon,

III) H (flagellar) antigens (phase 1) followed by a colon, and

IV) H antigens (phase 2, if present) (for example, *Salmonella* serotype IV 45:g, z_{51}:-)

For formulae of serotypes in *S.* bongori, V is still used for uniformity (for example, *S.* V 61:z_{35}:-) (Brenner *et al.*, 2000).

Historically, species names were arbitrarily given to serovars for convenient reasons in medical practice. To avoid possible sources of confusion, names indicating geographical origin of the first strain of the new serovar are now used. These names, wrongly considered as species names, were for this reason italicized. They are in fact without taxonomic status, used to name bacteria frequently isolated in human or veterinary medicine. These names are no longer

italicized. The first letter is a capital letter. In clinical practice the subspecies name does not need to be indicated as only serovars of subspecies *enterica* bear a name. The name *Salmonella* serotype Typhimurium or *Salmonella* Typhimurium may be used for routine practice. Serovars of other subspecies of *S. enterica* and those of *S. bongori* are designated only by their antigenic formula (Popoff and Le Minor, 1997). However, a simplified nomenclature is often preferred in diagnostic laboratories with the named serotypes of *Salmonella* being regarded as 'species', such as *S. dublin* or *S. typhimurium* (Quinn *et al.*, 1999).

2.2. Detection of *Salmonella*

The genus *Salmonella* consists of resilient microorganisms that readily adapt to extreme environmental conditions. Salmonellae actively grow within a wide temperature range between 8^0C and 45^0C and pH range of 4 to 8, with an optimum temperature of 37^0C and pH of 6.5 to 7.5. They do not survive at temperatures higher than 70^0C. They catabolize D-glucose and other carbohydrates (D'Aoust, 1997; Acha and Szyfres, 2001).

In general, 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). Funk *et al.* (2000) using one enrichment and plating media demonstrated that sensitivity of *Salmonella* detection increased with fecal sample weight, and ranged from 9 % for rectal swabs to 78 % for 25 gram samples on a study conducted on swine. The magnitude of the effect observed, including the modest sensitivity (0.75 - 0.78) of the largest sample weight category (25 grams), clearly has important implications for the design and interpretation of epidemiologic studies based on culture of *S. enterica* in swine herds.

2.2.1. Pre-enrichment in non-selective liquid medium

The test sample is initially inoculated into a non-inhibitory liquid medium to favour the repair and growth of stressed or sublethally-injured salmonellae arising from exposure to heat, freezing, desiccation, preservatives, high osmotic pressure or wide temperature fluctuations

(Andrews, W. H. 1989; D'Aoust, 1989). This step in the detection of *Salmonella* involves inoculation of buffered peptone water (also used as diluent) (ISO 6579, 1998) or lactose broth (FDA, 1998) with the test portion, and incubation at 35^0C or 37^0C 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 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) or1 ml mixture to 10 ml tetrathionate (TT) broth (FDA, 1998). The inoculated RV medium is incubated at 42^0C for 18 to 24 h (ISO 6579, 1998) or 24 ± 2 h at 42 ± 0.2°C (FDA, 1998), and selenite cystine medium at 35^0C to 37^0C for 18h to 24h (ISO 6579, 1998). The TT broth is incubated at 24 ± 2 h at 43 ± 0.2°C (FDA, 1998).

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 (TSB) for resuscitating damaged cells from heat-stressed, desiccated, or otherwise less than ideal samples. Other specimens containing high numbers of competing bacteria such as feces or ground meats may require highly selective media to prevent overgrowth by coliforms that can readily out-compete the *Salmonella* (Maddox, 2003). If *Proteus* species are a problem, the enrichment broths can be incubated at 43^0C, or sodium sulphathiazole added to the broths at 0.125 mg/100 ml. The host-adapted serotypes from swine and poultry are more fastidious than most of the other commonly isolated serotypes. They do not tolerate selenite broth or tertrathionate broth (Quinn *et al.*, 1999).

2.2.3. Plating out and identification

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.

According to FDA (1998), 3 mm loopful (10 µl) incubated TT broth and RV medium is streaked on bismuth sulfite (BS) agar, xylose lysine desoxycholate (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).

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).

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 nutrient 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 center 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

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) center. Colonies characteristic for *Salmonella* on the selective/indicator media are inoculated, singly in to 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. Salmonellae are oxidase negative and catalase positive, grow on citrate as the sole carbon source, do not hydrolyze urea and produce acid and gas from glucose in TSI. If the reaction in TSI and lysine decarboxylase broth is equivocal, further biochemical tests should be carried out or an identification system used such as API 20E (Analytab products) (D'Aoust, 1997; Quinn *et al.*, 1999). *Salmonella* generally are β-galactosidase, Voges-Proskauer and indole negative (ISO 6579, 1998).

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 detection of the presence of *Salmonella* O-, Vi- and H- antigens is tested by slide agglutination with the appropriate sera, from pure colonies and after auto-agglutinable strains have been eliminated (ISO 6579, 1998).

2.2.5. Typing of *Salmonella*

A typing method is defined as any method that can be used to differentiate bacteria beyond species level. Outbreak investigations and tracing of zoonotic bacteria among livestock and from livestock via food to man can be performed by the use of bacterial typing methods. These methods can subdivide a bacterial species into individual clonal lines, i.e. groups of bacteria produced by the continuous division of cells from the same ancestor (Olsen *et al.*, 1993).

There are phenotypic and genotypic typing methods. Originally, phenotypic typing methods, such as serotyping, phage typing and biotyping, were the only methods applied. These traditional methods still play a very important role in tracing of bacteria, and sporadic cases of salmonellosis, for example, will only be grouped to form a tentative outbreak if the isolates show identical serotyping, and where applicable, identical phage typing results. These methods still provide the basic, definitive background against which more advanced typing methods are applied (Olsen *et al.*, 1993).

2.2.5.1. Serotyping

Sereotyping 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 loopfull 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 al.*, 1999).

A 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 al.*, 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 *Salmonella*; 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* serovars have been described, and the number increases annually as new serovars are recognized (Wray and Davies, 2003).

2.2.5.2. Phage typing

Phage typing schemes for *Salmonella enterica* serovars are based on patterns of lysis produced by distinct phages isolated from a variety of sources (Olsen *et al.*, 1993; Quinn *et al.*, 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 labor 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 al.*, 1993). Phage typing has been used to subdivide isolates within serovars Typhi, Typhimurium, Enteritidis, Virchow, Hadar and Heidelberg. Although phage typing is essential for the subdivision of *Salmonella* serovars, the method can prove inadequate for serovars in which a small number of phage types predominate (Heuzenroeder *et al.*, 2004).

2.2.5.3. Antibiogram typing

Antimicrobial agents have been used in agriculture since the early 1950s to treat infections and improve growth and feed efficiency. The widespread use of antimicrobial agents in food animals is associated with increasing antimicrobial resistance in foodborne pathogens and subsequent multidrug-resistant bacterial infections in humans (Angulo *et al.*, 2004). In recent years, antimicrobial-resistant *Salmonella* strains have been isolated with increasing frequency (Gebreyes *et al.*, 2000). Increasing antimicrobial resistance in food borne pathogens may result in treatment failures, prolonged or more severe illness, increased hospitalization, and increased mortality (Angulo *et al.*, 2004). Resistance to antimicrobial agents is considered to be relatively unstable, because the majority of bacterial resistance factors are carried on plasmids that are often transferable between strains, and which may be dependent on selection pressure to be stably maintained. As a consequence, antibiograms are rarely used as the only typing method. The epidemiological importance is secondary to the implications for therapy and control. However, the results of these investigations can readily be used for epidemiological purposes. The spread of multiple resistant strains among livestock is often traced using the antibiogram as a typing method, preferably combined with other typing methods (Olsen *et al.*, 1993).

2.2.5.4. Genetic and molecular typing methods

Genotypic typing involves molecular techniques that enable DNA-based analyses of chromosomal or extra-chromosomal genetic elements, such as pulsed field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP) (Maslow *et al.*, 1993). Molecular typing is used when conventional methods fail to give sufficient discrimination between

isolates (Heuzenroeder *et al.*, 2004). Genetic typing methods involve plasmid profiling, plasmid restriction analysis, restriction analysis of the full genome and PCR-based typing. Bacterial genomes can be compared by electrophoretic separation of DNA-fragments generated in vitro by digestion with restriction endonuclease enzymes (Olsen *et al.*, 1993). Pulsed field gel electrophoresis has been in use for some considerable time and is the accepted gold standard adopted by organisations such as CDC in Atlanta (Heuzenroeder *et al.*, 2004). Plasmid profiling and plasmid restriction analysis has been extensively used for typing of *Salmonella*, often to fine-tune conclusions based on phage typing results (Olsen *et al.*, 1993).

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).

2.3.1. Distribution

Members of the genus *Salmonella* are ubiquitous pathogens found in humans and their livestock, wild mammals, reptiles, birds, and even insects (Davis *et al.*, 1990). As intestinal forms, the organisms are excreted in feces from which they may be transmitted by insects and other living creatures to a large number of places; they may also be found in water, especially polluted water (Jay, 2000). Although primarily intestinal bacteria, they are wide spread in the environment and commonly found in farm effluents, human sewage, and in any material subject to fecal contamination. Salmonellosis has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry, especially poultry and swine production (Wray and Davies, 2003).

Worldwide, *S.* Enteritidis is the most prevalent serotype, followed by *S.* Typhimurium. Changes in the relative frequency of serotypes can be observed over short period of time, sometimes within one or two years. Only a limited number of serotypes are isolated from man or animals

in a single region or country. The predominance of one or another can vary over time. Some serotypes, such as *S.* Enteritidis and *S.* Typhimurium, are found worldwide; in contrast, *S.* Weltevereden seems to be confined to Asia (Acha and Szyfres, 2001).

2.3.2. Host range

Salmonellae have a wide variety of domestic and wild animal hosts (Acha and Szyfres, 2001). All members of the genus are considered to be potentially pathogenic, although serovars may differ widely in their host range and the pathogenic syndromes that they produce. Some serovars appear to show a degree of host adaptation and primarily infect one animal species. They also tend to cause more severe illness than the other serovars. For example, *S.* Pullorum and *S.* Gallinarum infect poultry, *S.* Choleraesuis occurs in swine, and *S.* Dublin appears to have predilection for cattle, although occasional outbreaks of disease caused by this serovar occur in sheep. In contrast, *S.* Typhimurium affects all species of animals and is one of the most common causes of food poisoning in humans. The numerous other serovars are widely distributed, and the predominant serovar for an animal species in a country may vary over the year. Although the disease can affect all species of domestic animals, young animals and pregnant animals are the most susceptible. Many animals may also be infected without showing illness (Wray and Davies, 2003).

2.3.3. Sources of infection and transmission

2.3.3.1. Source of infection for animals

Infected animals are the sources of the organisms, which they excrete, and infect other animals directly (animal- to- animal transmission), or indirectly by contamination of the environment, primarily feed and water supplies. The farm animal may be infected in many ways: by animal-to- animal transmission, especially of host-adapted serovars; by contaminated animal feed; and by a contaminated environment (soil, birds, rodents, insects, water supplies). Non-species

specific serotypes also spread easily from one animal species to another and also to humans (Radostits *et al.*, 1994; Acha and Szyfres, 2001).

Feed contaminated by such ingredients as bone, meat, or fishmeal plays an important role as a vehicle of infection. Close contact between animals and the use of concentrated feed or ingredient that may be contaminated create conditions favorable to outbreaks (Acha and Szyfres, 2001). Feeds may be contaminated, during storage, by wild animals, especially rodents or birds. Flies and other insects have also been shown to be vectors of *Salmonella*. Contamination may also occur in the feed mill or during transport either to the mill or to the farm (Wray and Davies, 2003).

Farm building may became directly contaminated with *Salmonella* following outbreaks or indirectly contaminated from other sources such as contaminated water used for cleaning or from wild animals and birds. Dust in animal house may contain large number of microorganisms and may cause airborne transmission of *Salmonella* (Wray and Davies, 2003).

Salmonella may survive for long periods in infected feces and slurries. In most, uncomposted feces, *Salmonella* may survive for 3 to 4 months in temperate climates and for longer period in hotter climates (Wray and Davies, 2003).

Contaminated drinking water may facilitate the rapid spread of *Salmonella* among farm animals, which often defecate in their drinking water. Infection by such water may occur during flooding, contaminating the pasture (Wray and Davies, 2003). In developing countries, the source of infection is mainly the contaminated environment and water sources where animals crowd together (Acha and Szyfres, 2001).

Because *Salmonella* are often shed in large numbers in the feces, fecal-oral route is likely a major route for transmission of the organisms. However, other possibilities, such as infection via mucous membrane of the conjunctivae or upper respiratory tract are suspected (Quinn *et al.*, 1999; Wray and Davies, 2003). Aerosol or dust-borne transmission, need to be considered especially, where large group of animals are housed together. In poultry, ovarian transmission may be caused either directly or indirectly by *Salmonella* (Wray and Davies, 2003).

2.3.3.2. Source of infection for human beings

Animals are the reservoir of zoonotic salmonellae. Practically any food of animal origin can be a source of infection for humans. The most common vehicles are contaminated poultry, pork, beef, eggs, milk, and milk and egg products. Chickens, turkeys, geese, and ducks constitute the most important animal reservoir of asymptomatic *Salmonella* excreters in the human food chain (D'Aoust, 1998; Acha and Szyfres, 2001). Foods of vegetable origin contaminated by animal products, human excreta, or dirty utensils, in both commercial processing plants and household kitchens, have occasionally been implicated as vehicle of human salmonellosis. 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 being by direct or indirect contact to animals has been reported (Fey *et al.*, 2000; Hendriksen *et al.*, 2004).

Important contributing factors are inadequate cooking, slow cooling of the food, lack of refrigeration for many hours, and inadequate reheating before serving. Large outbreaks are invariably due to improper handling of food in restaurants and institutional dining facilities. Humans can also contract the infection directly from domestic animals or house pets, such as dogs, turtles, monkeys, hamsters, and others (Acha and Szyfres, 2001).

2.3.4. Carrier state

Because salmonellae are facultative intracellular organisms that survive in the phagolysome of macrophages, they can evade the bacteriocidal effects of antibody and complement. Thus, persistence of infection in animals and in the environment is important epidemiological features of salmonellosis. When an animal is infected with *S.* Dublin, for example, it may become a clinical case or an active carrier, passing organisms constantly or intermittently in the feces. It may also become a latent carrier with infection persisting in lymph nodes or tonsils but no salmonellae in the feces, or even a passive carrier which is constantly picking up infection from the pasture or the calf pen floor, but is not invaded so that when it is removed from the

environment the infection disappears. These animals probably multiply the salmonellae without becoming permanent carriers. 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). Carrier animals perpetuate the animal-to-animal cycle by means of their excreta or, in the case of fowl, through infected eggs (Acha and Szyfres, 2001).

Almost one-half of infected persons continue to excrete salmonellae one month after the symptoms have disappeared, and one in 20 still do so five month later. An individual who continues to excrete the organisms after one year is considered a carrier: the rate following typhoid is about 3 % and 0,5 % following nontyphoidal salmonellosis. An unknown fraction of people becomes carriers after asymptomatic infection: the median carriage rate of *Salmonella* among healthy persons in developing countries is about 0.1 % (Davis *et al.*, 1990).

2.4. Salmonellae in swine and pork

Salmonella infections in swine are of concern for two major reasons. The first is the clinical disease in swine (salmonellosis), and the second is that swine are susceptible to infections with a broad range of *Salmonella* serotypes constituting a potential source of human exposure and illness (Schwartz, 1993; Dickson *et al.*, 2003). Because of the frequency with which swine are infected with different types of salmonellae, pork products have often been a source of human infection (Acha and Szyfres, 2001). In swine, infection with a variety of serotypes is common but few serotypes are significant causes of disease. Clinical disease is usually caused by *S.* Choleraesuis, occasionally, *S.* Typhimurium or, rarely, *S.* Typhisuis is involved in localized epizootics. Infection with other serotypes rarely causes disease unless a naïve or compromised host ingests numerous organisms or a carrier becomes immunocompromised (Schwartz, 1993).

Swine represent important source of *Salmonella* serotypes causing disease in humans. In Denmark, The Netherlands and Germany it was estimated that 10-15%, 14-19% and 18-23%, respectively, of the salmonellosis cases in humans were attributable to pork (Hald and Wegener, 1990).

In swine, infection with a variety of serotypes is common, however, few serotypes are significant causes of disease (Schwartz, 1993). Pegram and associates (1981) isolated *S.* Saintpaul from one pooled sample of 10 mesenteric lymph nodes from swine slaughtered in Addis Ababa abattoir, Ethiopia. There is no any other information, which may show carriage state of *Salmonella* in Ethiopian swine.

Morgan and colleagues (1987) studied salmonellae isolated from swine cecum in Australia. The most frequent serotypes they identified were *S.* Derby (37.6%), *S.* Anatum (19.5%), *S.* Agona (12%), *S.* Give (11.3%), and *S.* Havana (10.5%). Typhimurium was only 2.2%. During the same study the most important carcass contaminant was *S.* Agona (31.2%), followed by *S.* Anatum (24.7%), *S.* Havana (18.2%), and *S.* Give (16.9%).

Letellier *et al.* (1999) conducted investigation on 5 porcine finishing units previously identified as contaminated by *Salmonella*. They identified 10 serotypes, with predominance of *S.* Derby (37.1%) and *S.* Typhimurium (34.1%) from serogroup B. Other serotypes of the serogroup B were *S.* Heidelberg (4.5%) and *S.* Schwartzengrund (0.8%). From serogroups C1, *S.* Ohio (8.3%) and *S.* Infantis (2.3%), from serogroup E, Anatum (10.6%) and others (2.4%).

The predominant serovars of *Salmonella* isolated from fecal, mesenteric lymph node, floor swabs of finishing barns and trucks, and cecal tissue samples of swine, in North Carolina were Typhimurium var. Copenhagen (33.2%), Derby (24.2%), Typhimurium (14.1%), Heidelberg (7.4%), and Infantis (3.9%), together comprising 82.5% of all isolates (Gebreyes *et al.*, 2000).

Rowe *et al.* (2003) studied 59 Irish farrow-to-finish swine herds and reported that 40% of positive herds were infected with *S.* Typhimurium only, while 27% were infected with *S.* Derby only and 23% were infected with both *S.* Typhimurium and *S.* Derby; serotypes London, Livingston and Infantis were each isolated from a single herd.

From samples collected on 90 swine farms in Alberta, Canada 98 were positive (Raie *et al.*, 2005). The most dominant serotypes isolated were *S.* Typhimurium (24.2%), Derby (22.0%) and Infantis (14.0%). Among 304 *Salmonella* serovars identified isolates 65.2%, 22.7%, 5.6%, 6.9%, 0.7% and 3.0% belonged to the serogroups B, C1, D1, E1, F and G2, respectively.

Isolation rate of *Salmonella* from pork compared with meat samples is high and stands only second to poultry. Contamination of pork carcasses is considerably higher than beef and ranges from 0.4 to 76.3%, with median of 16.2% (D'Aoust, 1989; Zhao *et al*., 2001). *S.* Infantis, *S.* Braenderup and *S.* Vejle were isolated from 16.4% of pork samples collected from supermarkets in Addis Ababa (Ejeta *et al*., 2004). Almost 98.1 % prevalence of *Salmonella* in raw pork with isolation of 34 serovars was reported from butcher shops in Mexico (Escartin *et al*., 1995), whereas 3.3 % in retail pork in USA (Zhao *et al*., 2001). The 10 most frequent serovars isolated in the former study were *S.* Agona, *S.* Derby, *S.* Anatum, *S.* Meleagridis, *S.* Enteriditis, *S.* Worthing, *S.* Give, *S.* Manhattan, *S.* Typhimurium, and *S.* Brandenburg (Escartin *et al*., 1995). A study conducted to determine the occurrence of *Salmonella* in carcasses in 12 slaughterhouses of five European countries demonstrated a 5.3 % overall prevalence. They demonstrated that the prevalence was higher during the warmer month of the year as compared to colder months (Hald *et al*., 2003).

Contamination of carcasses can easily result from rampant shedding at slaughter or fecal contamination during processing (Patterson and Isaacson, 2003). Morgan *et al*. (1987) demonstrated that the source of carcass contamination was primarily intestinal *Salmonella* infections and the extent of carcass contamination was determined by the number of *Salmonella* entering the abattoir in the intestine of slaughtered swine. According to Berends *et al*. (1997), live animals that carry *Salmonella* are 3–4 times more likely to end up as a positive carcass than *Salmonella*-free animals. Transport of swine to the slaughterhouse presents risk to the contamination of pork products with *Salmonella* serotypes. Contamination of animals in the slaughterhouse itself can also be important (Rajsch *et al*., 2003). A seven fold higher *S. enterica* isolation rate from swine necropsied at abattoir (39.9%) after 160 km transportation in clean, disinfected trailer than from those necropsied on the farm (5.3%) was demonstrated (Hurd *et al*., 2002).

Swine also shed antimicrobial- resistant *Salmonella* serotypes that pose threat to food safety. With increasing frequency, *Salmonella* isolates obtained from swine are resistant to one or multiple antimicrobials. A common resistance is to tetracycline, an antibiotic class used routinely in swine feed to improve feed efficiency. Resistance to several other antibiotics has commonly been found. Resistance against tetracycline (84.2%), ampicillin (47.6%), amoxicillin

and clavulanic acid (32%) and chloramphenicol (30.5%) was demonstrated among isolates of salmonellae from swine in USA (Gebreyes *et al.*, 2000; Rabsch *et al.*, 2003).

Salmonella Choleraesuis is very invasive and causes swine paratyphoid or necrotic enteritis. It occurs mostly in poorly managed herds living in poor hygienic conditions. Swine are particularly susceptible and experience epidemic outbreaks between 2 and 4 months of age, but the infection also appears in mature animals, almost always as isolated cases. The most frequent symptoms are fever and diarrhea. The infection usually originates from a carrier swine or contaminated feed. Infection of human by *S.* Choleraesuis is rare but important because it produce a severe disease syndrome. Swine can also suffer clinical disease in the form of enterocolitis caused by one of a number of serotypes, *S.* Typhimurium being the most notable (Acha and Szyfres, 2001; Rabsch *et al.*, 2003).

2.5. Pathogenesis

Salmonellae need to colonize the distal small intestine or colon to initiate enteric disease. The attachment of salmonellae is usually by fimbriae. Some strains producing enteritis and diarrhea appear capable of forming LT-like and ST-like enterotoxins and a cytotoxin. However, the diarrhea associated with salmonellosis is thought to be associated primarily with the inflammatory response, which stimulates local prostaglandin synthesis (Quinn *et al.*, 1999). The invasive strains replicate intracellularly but cause little mucosal damage or inflammation. They pass rapidly through the epithelial barrier and eventually proliferate in the lamina propria, they multiply within the macrophages of the liver and spleen as well as intracellularly. Any salmonellae in mononuclear phagocytes that are phagocytosed tend to survive within the phagocyte. Multiplication of the organisms in the body leads to a severe endotoxaemia. Invasive strains occur frequently in *S.* Typhi, *S.* Dublin and *S.* Typhimurium (Davies *et al.*, 1990; Quinn *et al.*, 1999).

Local host defense mechanisms prevent access of the invading *Salmonella* to the lymphatics and systemic circulation, so that infection with non-typhoidal strains is usually mild and self-limiting. However, severe acute and even death may occur in hosts impaired in humoral or

in cell-mediated immunity or with a compromised reticuloendothelial system. The acute gastroenteritis caused by many *Salmonella* serotypes is also associated with transient bacteremia (Davis *et al.*, 1990).

2.6. Clinical picture

2.6.1. In animals

The infection may or may not be clinically apparent. In the subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material briefly, intermittently, or persistently (Acha and Szyfres, 2001). The disease is most satisfactorily described as three syndromes classified arbitrarily according to severity as septicemia, acute enteritis, and chronic enteritis. There is no significant difference between infections caused by the different *Salmonella* serotypes (Radostits *et al.*, 1994). Although, there are several well-known clinical entities due to species-adapted serotypes, such as *S.* Pullorum or *S.* Abortusequi; other clinically apparent or inapparent infections are caused by serotypes with multiple hosts (Radostits *et al.*, 1994; Acha and Szyfres, 2001). Distinct differences exist between the clinical signs associated with porcine salmonellosis caused by different serotypes. *S.* Choleraesuis causes fever, diarrhea, and septicemia resulting in enterocolitis, pneumonia, meningitis, encephalitis, and chronic wasting while *S.* Typhimurium leads to fever and diarrhea (Roof *et al.*, 1993).

2.6.1.1. Septicemia

This is the characteristic form of the disease in newborn foals and calves and young swine up to four months old especially in the early stages of an outbreak. Affected animals show profound depression, dullness, prostration, high fever (40.5-42°C) and death within 24 to 48 hours (Radostits *et al.*, 1994).

2.6.1.2. Acute enteritis

This is the common form in adult animals of all species. There is a high fever (40-41°C) with severe fluid diarrhea, sometimes dysentery, and with tenesmus occasionally. The fever often subsides precipitously with the onset of diarrhea. The feces have a putrid smell and contain mucus, sometimes blood, fibrinous casts which may appear as complete tubular casts of intestine, and intestinal mucosa in sheets or casts. There is complete anorexia, but in some cases increased thirst. The pulse rate is rapid, the respiration is fast and shallow and mucosae are congested. Pregnant animals commonly abort. The case fatality rate without early treatment may reach 75%. In all species, severe dehydration and toxemia occur, the animal loses weight and strength rapidly, become recumbent and dies in 2 to 5 days. Newborn animals that survive the septicemic state usually develop severe enteritis with diarrhea becoming evident at 12 to 24 hours after the illness commences. If they survive this stage of the illness, residual polyarthritis or pneumonia may complicate the recovery phase (Radostits *et al.*, 1994).

2.6.1.3. Chronic enteritis

This is a common syndrome in swine and occurs occasionally in cattle and adult horses. It is also known as subacute enteritis. In calves there is intermittent or persistent diarrhea, with the occasional passage of spots of blood, mucus and firm fibrous casts, intermittent moderate fever (39°C), and loss of weight leading to emaciation. Although chronic enteritis may occur initially, it usually succeeds an acute episode (Radostits *et al.*, 1994).

2.6.2. In humans

The clinical pattern of salmonellosis can be divided into gastroenteritis, enteric fever (typhoid-like disease), bacteremia with or without focal extraintestinal infection, and the asymptomatic carrier state. Virtually any *Salmonella* serotype can cause any of these manifestations under appropriate conditions (eg. in a compromised host) and can persist afterward. However, certain serotypes are likely to be associated with a particular clinical syndrome; for example, *S.* Typhimurium, *S.* Enteritidis, and *S.* Newport with gastroenteritis; *S.* Typhi and the paratyphoid

serotypes with enteric fever; and *S.* Choleraesuis with bacteremia and local infection without antecedent gastrointestinal disturbance (Davis *et al.*, 1990).

Salmonellae of animal origin cause an intestinal infection in man characterized by a 6 to 72 hour incubation period after ingestion of the implicated food, and sudden onset of fever, myalgia, cephalgia, and malaise. The main symptoms consist of abdominal pain, nausea, vomiting, and diarrhea. Salmonellosis normally has a benign course and clinical recovery ensues in two to four days. The convalescent carrier may shed salmonellae for several weeks and, more rarely, for a few months. Conversely, the carrier state is persistent in infections due to *S.* Typhi or paratyphoid salmonellae. Although salmonellosis may occur in persons of all ages, incidence is much higher among children and the elderly. Dehydration can be serious (Acha and Szyfres, 2001).

2.7. Treatment

Salmonellas are sensitive to a number of antibiotics, for instance ampicillin, amoxicillin, chloramphenicol, gentamicin, trimethoprim-sulphonamide combinations, fluoroquinolones and nitrofuran derivatives. Bacterial resistance, however, has been found towards several antimicrobials (Seifert, 1996).

Early treatment with broad-spectrum antibiotic and with sulfonamides is highly efficient in preventing deaths and returning animals to normal function. In cases which are treated as soon as diarrhea with fever is observed, the cure rate is likely to be of the order of 100%, except in the case of foals and calves in which a fulminating septicemia is apt to defeat even the best treatment program (Radostits *et al.*, 1994).

There is a debate on the effect of antibacterial therapy for salmonellosis in animals as to a risk entailed in producing "carrier" animals and creation of drug-resistant strains to the bacteria. However, treatment is recommended for all sick animals using antibacterial agents, but many veterinarians will depend solely on supportive treatment, particularly the administration of fluids parenterally (Radostits *et al.*, 1994).

Zoonotic salmonellae usually heal without complications and the only treatment recommended is dehydrations and electrolyte replacement (Acha and Szyfres, 2001). In uncomplicated *Salmonella* gastroenteritis, patients should be monitored for fluid and electrolyte balance, as in any diarrheal disorder. Antimicrobial therapy does not reduce the duration and severity of symptoms and may in fact prolong convalescence and intestinal carriage of the infecting microorganism. However, some physicians treat infants and elderly persons who have acute gastroenteritis, to prevent complications. Patients with bacteremia, meningitis, enteric fever, or other extra intestinal infections require antimicrobial treatment (Davis *et al*., 1990).

2.8. Prophylaxis and control

Control is important to reduce both public health risk and economic losses. In animals, salmonellosis control consists of: (a) elimination of carriers, which is currently possible for pullorum disease and fowl typhoid by means of serologic tests; (b) bacteriologic control of feeds, mainly of such ingredients as fish, meat, and bone meal; (c) immunization; and (d) proper management of herds and poultry farms (Acha and Szyfres, 2001).

Hygienic premises, cleanliness, provision of non-contaminated feed and drinking water as well as appropriate feeding are important prerequisites for the prevention of salmonellosis. Infected and/or latently diseased animals have to be separated and treated. They can only return into the herd if they are found to be negative through bacteriological control. In poultry heat treatment of feed and screening of breeding flocks serologically and destruction of infected ones is practiced (Doyle and Cliver, 1990; Snyfers, 1990).

Given current conditions under which cattle and poultry are raised, transported, marketed, and slaughtered, as well as existing food processing practices, it is impossible to obtain salmonellae-free foods of animal origin. Control is currently based on protecting humans from infection and reducing its prevalence in animals. Veterinary meat and poultry inspection and supervision of milk pasteurization and egg production are necessary for consumer protection (Acha and Szyfres, 2001).

There are many approaches to controlling *Salmonella* in foods. The first way is to prevent entrance of the organism through contaminated raw materials. This is particularly important in ingredients, especially in foods of animal origin or those that may be contaminated by fecal excrement that do not receive a heat treatment that will kill *Salmonella* (Doyle and Cliver, 1990). An additional measure in controlling *Salmonella* in foods is to apply a heat treatment that would kill *Salmonella* during processing. Another important control measure is the education of food handlers, both in commercial establishments and in the home, about correct cooking and refrigeration practices for foods of animal origin, and about personal and environmental hygiene to prevent postprocessing contamination (Doyle and Cliver, 1990; Acha and Szyfres, 2001).

Immunization may be an important method for preventing human salmonellosis. Effective vaccines of different types are available in the market for prophylaxis of *S. Typhi* in humans (D'Aoust, 1997).

2.9. *Salmonella in* Ethiopia

2.9.1. Prevalence and serovar distribution

Infections caused by nontyphoid *Salmonella* in humans are increasingly frequent in developed and developing countries (Duwe, 1997; Defra, 2003). Nontyphoid salmonellae are important causes of diarrhoea in Ethiopia (Mache, 2002; Ashenafi and Gedebou, 1985; Mache *et al.*, 1997). Various workers isolated salmonellae from food animals in Ethiopia (Table 1). However, works on *Salmonella* in swine and pork are rare.

Table 1: *Salmonella* serovars isolated from different samples of food animals in Ethiopia

Species	Samples	No. of animals		Serovars isolated	Reference
		Examined	Positive		
Bovine	MLN*	280	6	*S.* Dublin, *S.* Bredeny	Pegram *et al.* (1981)
Porcine	MLN (pooled)	160	1	*S.* Saintpaul	"
Avian	Organs	-	77	*S.* Gallinarum	"
Bovine	feces (pooled)	235	5	*S.* Dublin, *S.* Muenchen	Nyeleti *et al.* (2000)
"	MLN (pooled)	235	9	*S.* Anatum, *S.* Dublin	"
"	AM**	235	23	*S.* Anatum, *S.* Dublin	"
"	DM***	235	28	*S.* Anatum, *S.* Dublin	"
Bovine	feces (pooled)	323	2	*S.* Mishmarhaemek	Alemayehu *et al.* (2003)
"	MLN (pooled)	323	3	*S.* Typhimurium, *S.* Enteritidis	"
"	AM	323	9	*S.* Mishmarhaemek , *S.* Typhimurium *S.* Guildford, *S.* Dublin	"
"	DM	323	10	*S.* Mishmarhaemek , *S.* Typhimurium *S.* Guildford, *S.* Dublin, *S.* Enteritidis	"
Camel	feces	270	18	*S.* Saintpaul, *S.* Muenchen, *S.* Kottbus, *S.* Havana, *S.* Heidelberg, *S.* Derby, *S.* Enteritidis, *S.* Anatum	Molla *et al.* (2004a)
"	MLN	9	9	*S.* Saintpaul, *S.* Braenderup, *S.* Muenchen, *S.* Typhimurium var. Copenhagen, *S.* Kottbus, *S.* Hadar, *S.* Bovismorbificans, *S.* Butantan, *S.* Infantis	"
"	liver	9	4	*S.* Saintpaul, *S.* Braenderup, *S.* Typhimurium var. Copenhagen, *S.* Kottbus, *S.* Hadar	"
"	spleen	9	7	*S.* Saintpaul, *S.* Typhimurium var. Copenhagen, *S.* Heidelberg, *S.* Braenderup, *S.* Infantis, *S.* Kottbus, *S.* Anatum, *S.* Butantan, *S.* Havana	"
"	AM	9	25	*S.* Saintpaul, *S.* Typhimurium, *S.* Braenderup, *S.* Muenchen, *S.* Kottbus, *S.* Hadar, *S.* Havana	"
"	DM	9	25	*S.* Saintpaul, *S.* Braenderup, *S.* Havana, *S.* Infantis, *S.* Muenchen, *S.* Kottbus	"
Ovine	Feces	180	3	*S.* Typhimurium, *S.* Enteritidis, *S.* Reading, *S.* Molla (2004) Heidelberg	

Species	Samples	No. of animals		Serovars isolated	Reference
		Examined	Positive		
"	MLN	104	8	*S.* Typhimurium var. Copenhagen, *S.* Typhimurium, *S.* Reading, *S.* Give, *S.* Heidelberg, *S.* Niederoderwitz	"
"	Liver	104	2	*S.* Typhimurium, *S.* Give	"
"	AM	104	2	*S.* Typhimurium, *S.* Heidelberg	"
"	Spleen	104	1	*S.* Typhimurium,	"
Caprine	Feces	100	2	*S.* Typhimurium, *S.* Poona	Molla (2004)
"	MLN	100	2	*S.* Poona	"
Ovine	Feces	47	1	*S.* Kottbus	Woldemariam *et al.*, 200
"	Liver	47	2	*S.* Infantis	"
"	AM	47	5	*S.* Infantis, *S.* Braenderup	"
"	DM	47	2	*S.* Kingbawa, *S.* Infantis	"
Caprine	Feces	60	2	*S.* Infantis, *S.* Kottbus	Woldemariam *et al.*, 20
"	MLN	60	7	*S.* Zanzibar, *S.* Infantis, *S.* Butantan, *S.* Typhimurium, *S.* Kingbawa, *S.* Anatum	"
"	Spleen	60	2	*S.* Infantis, *S.* Butantan	"
"	Liver	60	3	*S.* Butantan, *S.* Braenderup	"
"	AM	60	2	*S.* Butantan, *S.* Infantis	"
"	DM	60	2	*S.* Hadar, *S.* Infantis, *S.* Butantan	"

*mesenteric lymph nodes, **abdominal muscles, ***diaphragmatic muscle

Various serovars of *Salmonella* were isolated at high prevalence from samples of meat (Ibrahim *et al.*, 2003; Molla and Mesfin, 2003; Ejeta *et al.*, 2004; Zewdu 2004), ready to eat foods (Zewdu, 2004; Tegegne and Asheneafi, 1998) and other food items in Ethiopia (Zewdu, 2004) indicating the potential of human infections through consumption of raw or inadequately cooked food and cross contamination of cooked food (Table 2).

Table 2: *Salmonella* isolated from animal products in Ethiopia

Sample type	No. of samples		serotypes isolated	Reference
	Examined	Positive		
Raw 'Kitfo'	50	21	ND	Tegene and Ashenafi (1998)
Minced beef	330	26	*S.* Anatum, *S.* Dublin, *S.* Saintpaul	Nyeleti *et al.* (2000)
Chicken meat and giblets	301	54	*S.* Braenderup, *S.* Anatum, *S.* Saintpaul, *S.* Uganda	Tibaijuka *et al.* (2002)
Chicken meat and giblets	378	80	*S.* Braenderup, *S.* Typhimurium var. Copenhagen, *S.* Anatum, *S.* Kottbus and *S.* Typhimurium	Molla and Mesfin (2003)
Minced beef	160	23	*S.* Infantis, *S.* Braenderup, *S.* Anatum, *S.* Bovismorbificans, *S.* Vejle, *S.* Dublin, *S.* Saintpaul	Ejeta *et al.* (2004)
Mutton	85	12	*S.* Infantis, *S.* Braenderup, *S.* Anatum, *S.* Bovismorbificans	"
Pork	55	9	*S.* Infantis, *S.* Braenderup, *S.* Vejle	"
Chicken meat	208	29	*S.* Newport, *S.* Braenderup, *S.* Hadar, *S.* Typhimurium, *S.* Kentucky, *S.* Anatum	Zewdu (2004)
Pork	194	22	*S.* Newport, *S.* Infantis, *S.* Kottbus	"
Mutton	212	23	*S.* Newport, *S.* Hadar, *S.* Typhimurium, *S.* Kentucky, *S.* Anatum	"
Minced beef	142	12	*S.* Newport, *S.* Typhimurium, *S.* Infantis, *S.* Kentucky, *S.* Anatum, *S.* Saintpaul	"
Fish	128	3	*S.* Newport	"
Cheese	190	4	*S.* Newport	"

ND = not determined

Nontyphoidal salmonellosis is one of the most important foodborne diseases throughout the world. Different workers in Ethiopia investigated prevalence, serotype/serogroup distribution of *Salmonella* in humans (Table 3).

Table 3: *Salmonella* serovars serogroups isolated from humans in Ethiopia

Sample type	No. of samples		Serotypes/serogroups	Reference
	Examined	Positive		
Blood, stool			Serogroup A, B, C, E, *S.* Typhi	Gedebou and Tassew (1981)
Stool	1000	45	Serogroup A, C, B, D, E, *S.* Typhi	Ashenafi and Gedebou (1985)
Stool	700	45	Serogroup A, B, C, D, E, *S.* Typhi	Mache *et al.* (1997)
Stool	300	18	*S.* Anatum, *S.* Dublin, *S.* Meleagridis	Nyeleti *et al.* (2000)*
Stool	384	59	Serogroups A, B, C, D, E, *S.* Typhi	Mache (2002)
Stool	68	5	*S.* Newport	Zewdu (2004)*

* non-clinical samples

2.9.2. Antimicrobial resistance

There is an increase in the proportion of antimicrobial resistant salmonellae isolated from animals and humans in Ethiopia. Pegram *et al.* (1981) tested all salmonellas recovered from farm livestock, abattoir and bone factory for antimicrobial resistance. They found all isolates to be fully sensitive to ampicillin, chloramphenicol, tetracycline, kanamycin, sulphonamides, streptomycin, trimethoprim and furazolidone. Similarly, all isolates obtained from diarrheal outpatients in Addis Ababa were sensitive to gentamicin, polymyxin B and trimethoprim-sulphamethoxazole. About 69% were sensitive to all drugs tested (Ashenafi and Gedebou 1985).

Works conducted later on animal and human isolates indicated higher resistance of salmonellae to antimicrobials. Molla *et al.* (2003b) conducted research on 80 *Salmonella* isolates from chicken carcasses and giblets from processing plants at Debre Zeit and supermarkets in Addis

Ababa. They indicated that 65.7% were resistant to one or more antimicrobials, while 52.5% displayed multiple-drug resistance. Similarly, among 45 isolates obtained from 700 diarrheal stool samples from adult outpatients in Addis Ababa, 71.1% were resistant to tetracycline, 68.9% to ampicillin, 60.7% to cephalothin, 57.8% to trimethoprim-sulphamethoxazole, 53.9% to kanamycine, 46.7% to chloramphenicol (Mache *et al*., 1997).

Salmonella isolates from different sources in Ethiopia were resistant to commonly used antimicrobials. Out of 80 isolates from chicken carcasses and giblets 51.2% were resistant to sulfisoxazole, 45% to amoxicillin-clavulanic acid and ampicillin, 41.2% to tetracycline and 30% to chloramphenicol (Molla *et al*., 2003b). Among 59 isolates obtained from pediatric diarrheal outpatients in Jimma, Ethiopia, 59.3% were resistant to tetracycline and ampicillin, 40.7% to trimethoprim-sulfamethoxazole, 35.6% to chloramphenicol (Mache, 2002). Molla *et al*. (2004b) investigated antimicrobial resistance patterns of 116 *Salmonella* serotypes isolated from apparently healthy slaughtered camels in Ethiopia to 17 antimicrobials. Fifty-two (44.8%) of the *Salmonella* isolates were resistant to one or more antimicrobials: streptomycin (35.3%), sulfamethoxazole (25.0%), ampicillin (24.1%), trimethoprim (22.4%), trimethoprim-sulfamethoxazole (18.9%), tetracycline (12.9%). Relatively high level of resistance was documented for cephalotin (Mache, 2002; Molla, 2004).

3. MATERIALS AND METHODS

3.1. Study area

The study was undertaken at Addis Ababa abattoir. Addis Ababa lies in the central highlands of Ethiopia at an altitude of 2500 meters above sea level. The average annual temperature is 21°C with relative humidity varying between 70% to 80% during the rainy season and 40% to 50% during the dry season. The area receives an average of 1800 mm rain annually (CSA, 2001). Bacteriological analysis of the samples was conducted at the Microbiology Laboratory of Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Addis Ababa abattoir slaughters on average 18 swine once a week in the afternoons. The room where swine slaughtered was used only for swine slaughter. The slaughtering process involved stunning with electric shock followed by bleeding. The carcasses were then immersed in a scalding tank before being placed on a cradle for dehairing. Scalding was carried out in an open scalding tank and dehairing was conducted by shaving manually using knife. After raising the carcass to the processing rail evisceration and carcass splitting takes place. Carcasses were splitted manually and thereafter they were flamed singed to remove any remaining hair. Finally they were washed with tap water from a hand-held hose. Overhead rails were used from bleeding area to completion of the process. About 45 people were involved in the slaughtering operations and swine were slaughtered in one line. Since chilling facilities were not available for storage, carcasses stay hanging at the end of slaughter line until they were distributed in the next morning to the traders.

3.2. Study animals

The study was carried out on 278 apparently healthy slaughtered swine at Addis Ababa abattoir, slaughtered between October 2004 and February 2005. Both sexes and various age groups of swine were slaughtered during the study period. The swine originated mainly from farms in Addis Ababa and towns around the capital (Debre Zeit, Modjo, Zeway and Nazareth). Animals

were transported on trucks, in small groups, from farms to the slaughterhouse and were kept in concrete floored, open-air holding pens for an average of 3 hours without feed and water.

3.3. Study design

The study was cross-sectional observational study involving 278 swine slaughtered between October 2004 and February 2005 at Addis Ababa abattoir. Animals were selected with simple random sampling using the animal's slaughter order. On days in which manageable number of swine upto 15 were slaughtered, all the animals slaughtered that day were sampled.

The variable of interest considered as an output variable versus risk factors at the abattoir was carcass bacteriological status. The explanatory variables considered at the abattoir were animal cecal content, mesenteric lymph nodes and scalding water bacteriological status, and scalding water temperature.

3.4. Sampling

Samples were collected during 19 weekly visits to the slaughterhouse. During the sampling period a total of 323 swine were slaughtered. Addis Ababa abattoir slaughters about 1000 swine a year.

3.4.1. Sample size

The sample size was determined depending on the expected prevalence of *Salmonella* infection and the desired absolute precision adjusted to the average number of animals slaughtered annually at the slaughterhouse by the following formula (Thrusfield, 1995):

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

Where n is the sample size, based on an infinite population obtained from the formula below, and N is the size of the study population.

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

Where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

As there was no previous study on prevalence of *Salmonella* in swine in Ethiopia, 50% expected prevalence was used to estimate the sample size. Adjustment was necessary because sample size calculated based on an infinite population gave a result 5% or more of the study population (Thrusfield, 1995). Using desired 95% confidence interval, 5% precision and 50% expected prevalence the number of slaughter swine needed to demonstrate prevalence of *Salmonella* was 278.

Cecal content and mesenteric lymph nodes were collected from each 278 sample slaughter swine while carcass swab was collected from 277 animals due to withdrawal of one carcass from the slaughter line due to total condemnation of the carcass. Scalding water samples were collected on 16 occasions.

Table 4: Type and number of samples collected

Type of sample	Number of samples	Source of sample
Cecal content	278	Slaughtered swine
Mesenteric lymph nodes	278	"
Carcass swab	277	"
Scalding water	16	Scalding tank
Total samples	849	

3.4.2. Sampling procedures

On days in which manageable number of swine were slaughtered, all the animals slaughtered that day were sampled. If they were large in number, sample animals were selected randomly by their slaughter order. Cecal content, mesenteric lymph nodes and carcass swab samples were collected from each selected apparently healthy slaughtered swine in separate sterile sample containers.

The mesentery, with the lymph nodes attached, was removed from the intestines with scissors disinfected in 70% ethanol. It was brought to the laboratory in separate sterile plastic bags (or aluminum foil). Cecal content was collected from cecum in sterile universal bottle through a puncture made with scalpel blade scrubbed in 70 % ethanol. Carcass swabs were collected from three sites of one side of each sample carcass. These samples were collected by rubbing buffered peptone water (BPW) (AES laboratoire, Cedex, France) moistened sterile sponge swabs of approximately 78mm X 38mm over approximately 10cm X 10cm area of carcass at the ham, flank (belly) and jowl. The sponge swabs were moistened with approximately 10 ml BPW at the time of sample collection. Swabbing of carcass was conducted holding the swabs with sterile gloves. After swabbing, the swabs were returned to their original sterile plastic cases. Carcass swab samples were collected at the end of slaughtering process after they were washed with water. Scalding water samples were collected using sterile universal bottles during the slaughtering operation. Scalding water temperature was recorded for 12 sampling days taken during slaughter activities. Samples were transported in cool boxes on ice to the laboratory and processed within 24 hours of collection. When they were not processed within 24 hours they were stored in refrigerator at 4°C for a maximum of 7 days.

3.4.3. Sample processing

In the laboratory, the lymph nodes were freed from surrounding tissues and 25 gram was weighed, passed over flame to disinfect the surface, and cut into small pieces on a sterile plate using sterile scalpel blade. The minced lymph nodes were put into sterile stomacher bags and 225 ml BPW was added and homogenized for 2 minutes with a stomacher (Seward Stomacher

400, London, UK) at high speed. Twenty five grams of cecal content was weighed on sterile aluminum foil and was put in sterile flasks of 500 ml capacity. About 225 ml BPW was added and agitated manually to disperse the content. Each carcass swab was agitated manually with fingers in an additional 15 ml BPW while they were in their original plastic bags. Twenty five ml of scalding water was measured with sterile syringe and was put into 500 ml capacity flask and 225 ml BPW was added. Whenever samples were found less than 25 gm, BPW was added to the samples in 1:9 ratio.

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 detection of *Salmonella* necessitates different successive stages, which are described below. The bacteriological media used in different stages were prepared according to the manufacturer's recommendations (Appendix I). All the samples were processed and analyzed separately.

3.5.1. Pre-enrichment

Processed samples in appropriate amount of BPW (1:9) were incubated for 16 to 20 hours at 37°C. Mesenteric lymph node samples were incubated while they were in stomacher bags whereas cecal content and scalding water samples were incubated in flasks. Carcass swabs were incubated in the plastic bags.

3.5.2. Selective enrichment

Rappaport-Vassiliadis (RV) (Difco™, Becton Dickinson, USA) and selenite broth (SB) (Difco, Becton Dickinson, USA, France) media were used for selective enrichment. One ml incubated pre enrichment broth was transferred aseptically into 10 ml of SB and was incubated at 37°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°C for 18 to 24 hours.

3.5.3. Selective plating and identification

A loopful of inoculum from each RV and SB cultures were streaked onto brilliant green lactose sucrose (BPLS) agar (Merck, Darmstadt, Germany) and xylose lysine desoxycholate (XLD) agar (AES laboratoire, Cedex, France) plates. The inoculated plates were incubated at 37°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 BPLS agar give an alkaline reaction and have red colonies while on XLD medium they appear as pink colonies with or without black center. However, many cultures of *Salmonella* on XLD medium may produce colonies with large, glossy black centers or may appear as almost completely black colonies (ISO 6579, 1998; FDA, 1998; Quinn *et al.*, 1999).

3.5.4. Biochemical tests

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 very center 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 ± 1°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 agar slants (BBL™, Becton Dickinson, USA) and Rambach® agar (Merck, Darmstadt, Germany) plates for further characterization. The inoculated Rambach® agar plates were incubated at 37°C for 24 hours (Merck, 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) and sent to Health Canada, Office International des Epizooties (OIE)

Reference Laboratory for Salmonellosis for serotyping, phage typing and antimicrobial sensitivity tests. One isolate from each positive sample was sent for serotyping phage typing and antimicrobial resistance testing.

3.5.5. *Salmonella* serotyping

Serotyping of the *Salmonella* isolates was carried out at the Health Canada Office International des Epizooties (OIE) Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada. 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* serovars as listed by Popoff and Le Minor (1997) were used to name the serovars.

3.5.6. Phage typing

Phage typing of *Salmonella* isolates was done based on the sensitivity of a particular isolates to a series of bacteriophages at appropriate dilutions. This was carried out at the Health Canada Office International des Epizooties (OIE) Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada. The standard phage typing technique described by Anderson and Williams (1956) was employed throughout this investigation. Strains that did not conform to any recognized phage type were considered atypical (RT). Strains which did not react with any of the typing phages were considered untypable (UT). *Salmonella* Enteritidis strains were phage typed with typing phages obtained from the Central Public Health Laboratory, Colindale, London, United Kingdom (Ward *et al.*, 1987).

3.5.7. Resistance to antimicrobial agents

The *Salmonella* isolates were tested for susceptibility to 24 selected antimicrobial drugs. The test was carried out at the Food Microbiology Laboratory, Laboratory Services Division, Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada. The antimicrobial resistance tests of *Salmonella* strains were carried out with the agar dilution method.

The standards and reference strains described by National Committee for Clinical Laboratory Standards (NCCLS, 1999) for antimicrobial susceptibility tests of bacteria isolated from animals and humans were employed throughout the test. Briefly, the strains were grown to 0.5-1.0 McFarland density in Mueller Hinton (MH) broth (Difco, Detroit, USA) and replica plated using a Cathra Replicator (Brown and Washington, 1978) onto MH agar plates (Difco, Detroit, USA) containing antimicrobials at the concentration listed in Table 5. To determine resistance to florfenicol, Aquaflor® containing 50% florfenicol was dissolved in dimethylformamide and added stock solution containing 20 mg ml Aquaflor to MH agar to obtain plates with 16 µg ml of florfenicol. Isolates which were resistant to one or more antimicrobials considered as resistant strains and isolates which were resistant to two or more antimicrobials considered as multi-drug resistant strains. Intermediately resistant isolates were considered susceptible for practical purposes.

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

		Antimicrobial break point concentrations[a]	
		Susceptible at	Resistant at
Antimicrobial	Abbreviations	≤ µg/ml	≥ µg/ml
Amikacin	Amk	16	ND[b]
Ampicillin	Amp	ND	32
Amoxicillin clavulanic acid	Amc	ND	64/16[c]
Apramycin	Apr[d]	ND	32[e]
Carbadox	Crb[d]	ND	30[f]
Cephalothin	Cef	ND	32
Ceftriaxone	Cro	8	ND
Ceftiofur	Ctf	ND	8
Cefoxitin	Fox	ND	32
Chloramphenicol	Chl	ND	32
Ciprofloxacin	Cip	0.125[g]	ND
Florfenicol	Fen[d]	ND	16[h]
Gentamicin	Gen	ND	16
Kanamycin	Kan	ND	64
Nalidixic acid	Nal	ND	32
Neomycin	Neo[d]	ND	16[i]
Nitrofurantoin	Nit	ND	64
Spectinomycin	Spt[d]	ND	64[j]
Streptomycin	Str[d]	ND	32[k]
Sulfisoxazole	Sul	ND	521
Sulfamethoxazole/trimethoprim	Sxt	ND	76/4
Tetracycline	Tet	ND	16
Tobramycin	Tob	ND	8
Trimethoprim	Tmp	ND	16

The breakpoint concentrations to determine susceptible, intermediate and/or resistance were those specified by the NCCLS standards M31-A and M100-S12.

ND –not done.

The strains were considered resistant when growing on agar plates with amoxicillin/clavulanic acid at 64/16 µg/ml.

There are no interpretative standards specified by the NCCLS standards M31-A and M100-S12 for apramycin, carbadox, florfenicol, neomycin, spectinomycin and streptomycin.

Strains were considered to be resistant to apramycin, neomycin, spectinomycin and streptomycin at 32, 16, 64, and 32 µg/ml, respectively.

The strains were considered to be resistant to carbadox, a veterinary growth promoter for pigs, at 30 µg/ml.

A 0.125 µg/ml of ciprofloxacin concentration determines reduced sensitivity to ciprofloxacin.

Strains were considered to be resistant to florfenicol at the level of 16 µg/ml.

Strains were considered to be resistant to nitrofurantoin at 64 µg/ml; human urinary tract isolates are considered to be resistant to nitrofurantoin at 128 µg/ml (NCCLS, M100-S12).

3.6. Data management and analysis

The data were entered and managed in MS Excel and analysis was conducted using Stata version 7 (Stata Corporation, 2001), and SPSS release 11.5.0 (SPSS, 2002).

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 cecal content and or mesenteric lymph node sample was culture positive for *Salmonella*.

The data were analysed by comparing proportions using Pearson's chi-square test and multiple logistic regression analysis. The difference in serovars distribution in CC and MLN and difference in distribution of individual serovars in CC and MLN was compared using Pearson's chi-square test. In case of matched samples (cecal-lymphnode) McNemar's chi-square test was used (Thrusfield, 1995). For association of risk factors considered in the abattoir with carcass contamination, multiple logistic regression analysis was used. The explanatory variables

Table 6: Prevalence of *Salmonella* in different samples of slaughter swine in Addis Ababa

Sample	Number of Samples		
	Examined	Positive (%)	95% Confidence Interval
Mesenteric lymph nodes	278	99 (35.6)	30.0 - 41.2
Cecal content	278	63 (22.7)	17.8 - 27.6
Carcass swab	277	11(4)	1.7 - 6.3
MLN and/or CC	278	120 (43.2)	37.3-49.0
Scalding water	16	-	

Association of carcass contamination with MLN and CC *Salmonella* status was assessed using multiple logistic regression analysis, Table 7. Only CC was found associated with carcass contamination. The best fitted model to predict carcass contamination was the model which included only CC. A simple logistic regression yielded an odds ration of 6.7 for the factor CC status. Therefore, carcasses of animals which had *Salmonella* in their cecal content, were 6.7 times (OR=6.7, 95 % CI [1.90 - 23.76]) more likely to be contaminated with *Salmonella* compared to those which were negative. Moreover, carcass contamination with *Salmonella* was not observed on slaughter days, which were bacteriologically negative for cecal contents. A slaughter day was considered positive for CC if at least one CC sample was found positive. Nine out of 11 animals positive for carcass contamination were positive either for their cecal content or mesenteric lymph nodes and 7 out of 11 of these animals each were positive for cecal content and mesenteric lymph nodes.

considered (CC and MLN *Salmonella* status) were first separately analyzed to see their association with the outcome of the bacteriological test for carcass swab. Both CC and MLN *Salmonella* status and their interaction were then included in the logistic regression model as explanatory variables for carcass contamination because their P values were low (<0.2). Bacteriological result of scalding water sample was dropped from the analysis because none of the samples were found positive for *Salmonella*.

4. RESULTS

This study was conducted on 278 apparently healthy slaughter swine at Addis Ababa abattoir, from October 2004 to February 2005 with objectives of determining the prevalence of salmonellae, identify serovar distribution and antibiotic resistance, and identify some of the risk factors associated with carcass contamination at slaughter. Bacteriological examination was conducted on 278 cecal content (CC), 278 mesenteric lymph nodes (MLN), 277 carcass swab (CS) and 16 scalding water (SW) samples.

4.1. Prevalence of *Salmonella*

Out of a total of 278 animals examined for bacteriological status of *Salmonella*, 120 (43.2%) were positive. In this study, an animal was considered *Salmonella* positive when it was bacteriologically positive either for its CC and/or MLN. Carcass status was considered rather an indicator of contamination. Of the samples examined, 99 mesenteric lymph node samples (35.6%) and 63 (22.7%) cecal content samples were *Salmonella* positive. Only 11 (4.0%) of 277 carcass swab samples were found positive for *Salmonella* (Fig. 1). None of the 16 scalding water samples were positive for *Salmonella*. The temperature of the scalding water during activity was between 65 and 76°C with an average of 72°C. Out of the total 849 samples collected during the study, including scalding water, 173 (20.4%) were culture positive for *Salmonella* (Table 6).

Salmonellae were found more prevalent in mesenteric lymph nodes (35.6%) compared to cecal content (22.7%) ($\chi 2 = 11.3$; $P<0.001$). Results of paired lymph node and cecal content cultures were strongly associated (McNemar's $\chi 2 = 15.7$; $p<0.001$). Out of a total of 120 positive animals, 42 were culture positive for both MLN and CC samples.

Table 7: Association of *Salmonella* status of cecal content and mesenteric lymph nodes with carcass contamination

Factor	Odds Ratio	Std. Err.	z	P-value	[95% Conf. Interval]	
CC	8.210526	8.449433	2.05	0.041	1.09245	61.70785
MLN	2.836364	2.871028	1.03	0.303	0.39008	20.62369
CC*MLN	0.465188	0.624982	-0.57	0.569	0.03342	6.47472

Salmonella was recovered from carcass swabs on 8 slaughter days of 19 visits. Four of the animals, which were positive for carcass sample, had the same serovar in their cecal content. Other 4 carcasses contaminated were slaughtered on the same day with other animals harboring the same serovars in their gut. The remaining three isolates contaminating carcasses were not isolated either from the same animal or animals slaughtered the same date (Table 8).

Table 8: Serovar distribution in samples of carcass positive animals

Animal no	Sampling date	Serovar from CC	Serovar from MLN	Serovar from CS	Same serovar from other animals*
30	12/10/04	S. Hadar	-	S. Hadar	present
69	09/11/04	S. Eastbourne	S. Eastbourne	S. Eastbourne	present
70	09/11/04	-	-	S. Eastbourne	present
150	14/12/04	S. Saintpaul	S. Saintpaul	S. Trough-O-eb-1,2	not present
171	21/12/04	-	S. Typhimurium	S. Eastbourne	present
228	11/01/05	S. Typhimurium var. Copenhagen	S. Typhimurium var. Copenhagen	S. Hadar	present
258	25/01/05	-	S. Newport	S. Havana	not present
261	25/01/05	S. Anatum	S. Newport	S. Anatum	present
280	01/02/05	-	-	S. 1:6,8:-:enx	present
281	01/02/05	S. 1:6,8:-:enx	-	S. 1:6,8:z10:-	not present
309	08/02/05	S. Kentucky	S. Kentucky	S. Kentucky	present

*presence of similar serovar in samples collected from other pigs slaughtered on the same date

Antimicrobial resistance patterns of serovars isolated from carcass swabs were identical to similar serovars isolated from other related samples (CC, MLN) of the same animal or animals slaughtered the same date, with the exception of one isolate (*S.* Kentucky) (Table 9).

Table 9: Antibiogram of *Salmonella* serovars isolated from carcasses and related samples

Animal No.	Serovars from carcass swab	Same serovars from other samples*	Antibiogram of isolates	
			CS isolates	Other samples
30	*S.* Hadar	*S.* Hadar	NitStrTet	NitStrTet
69	*S.* Eastbourne	*S.* Eastbourne	None	None
70	*S.* Eastbourne	*S.* Eastbourne	None	None
159	Rough		None	
171	*S.* Eastbourne	*S.* Eastbourne	None	None
228	*S.* Hadar	*S.* Hadar	NitStrTet	NitStrTet
258	*S.* Havana		None	
261	*S.* Anatum	*S.* Anatum	None	None
280	*S.* 1:6,8:-:enx	*S.* 1:6,8:-:enx	NitStrTet	NitStrTet
281	*S.* 1:6,8:z10:-		NitStrTet	
509	*S.* Kentucky	*S.* Kentucky	AmpAmeCipNal	AmpAmeCefCipNalStrSulTet AmpAmeCipGenNalSptStrSulTet

*Same serovars from CC and MLN of the same animal or animals slaughtered the same date

One hundred and twenty two animals were positive at least in one sample when carcass swab was considered in addition to MLN and CC. Out of these 122 animals 46 were positive in at least 2 samples, while 42 were positive both for CC and MLN. Only 5 animals, however, were found positive for all the 3 samples. Fifty seven swine were identified positive only for MLN while 21 swine were identified positive by CC only. Both MLN and CC samples were positive for 42 animals.

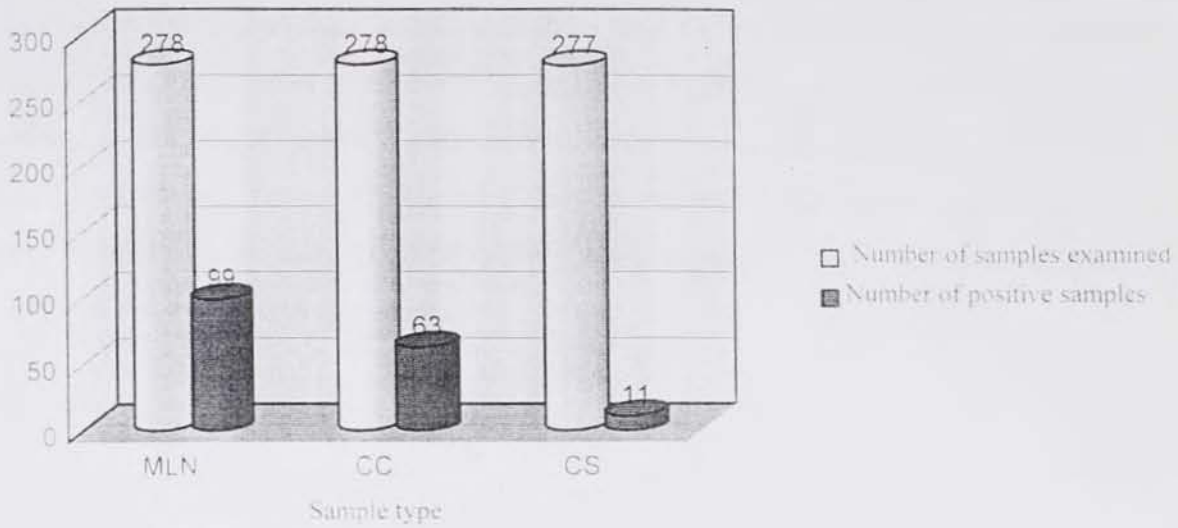


Fig 1: *Salmonella* positive samples from swine at Addis Ababa slaughterhouse

4.2. Serotypes identified

Among 173 isolates of *Salmonella* recovered during the study, 166 (96%) were identified as specific serovars, while for 7 (4%) isolates specific serovar designation was not possible because they lacked some of the somatic and/or flagellar antigens. The 166 serovar-identified isolates were composed of 17 serotypes, Table 10. Most of the isolates belonged to 3 serovars. The three most frequent serotypes identified were *S.* Hadar (27.2%), *S.* Eastbourne (22.5%) and *S.* Saintpaul (21.4%). About 71.1 % of the isolates identified in this study belonged to these serotypes. Less frequent serovars recovered include *S.* Typhimurium (5.2%), *S.* Typhimurium var. Copenhagen (3.5%) and *S.* Kentucky (2.9%). To our knowledge this is the first report of serovars *S.* Amager and *S.* Tarshyne in Ethiopia.

Distribution of the top four serovars in CC and MLN was significantly ($P = 0.0021$) different. The distribution of serotypes in different samples showed that *S.* Hadar (41.3%), *S.* Eastbourne (23.8%) and *S.* Saintpaul (15.9%) were the most dominant serotypes in CC where as *S.* Saintpaul (27.3%), *S.* Eastbourne (21.2%), *S.* Hadar (19.2%) and *S.* Typhimurium (9.1%) were the dominant serotypes in MLN, according to their frequency of occurrence (Table 10).

Distribution of individual serotypes also varied significantly between sample types. *S.* Saintpaul was more ($P = 0.005$) prevalent in MLN (75.0%) than CC (37.0%). *Salmonella* Infantis and *S.* Meleagridis were isolated only from CC. *Salmonella* Typhimurium, *S.* Enteritidis, *S.* Amager, *S.* Kottbus, *S.* Braenderup, and *S.* Tarshyne were isolated only from MLN, while *S.* Havana was isolated only from CS. *Salmonella* Hadar, *S.* Eastbourne and *S.* Kentucky were isolated from all the three samples. *Salmonella* Choleraesuis, the most important cause of clinical salmonellosis in swine was not detected in this study.

Table 10: *Salmonella* serotypes and their distribution in different samples isolated from slaughter swine in Addis Ababa

Serovar	Sample type and number of serovars identified by source			Total	%
	CC	MLN	CS		
S. Hadar	26	19	2	47	27.2
S. Eastbourne	15	21	3	39	22.5
S. Saintpaul	10	27	-	37	21.4
S. Typhimurium	-	9	-	9	5.2
S. Typhimurium var. Copenhagen	2	4	-	6	3.5
S. Kentucky	2	2	1	5	2.9
S. Enteritidis	-	4	-	4	2.3
S. Newport	1	3	-	4	2.3
S. Anatum	2	-	1	3	1.7
S. Amager	-	2	-	2	1.2
S. Infantis	2	-	-	2	1.2
S. Kottbus	-	2	-	2	1.2
S. Muenchen	1	1	-	2	1.2
S. Braenderup	-	1	-	1	0.6
S. Havana	-	-	1	1	0.6
S. Meleagridis	1	-	-	1	0.6
S. Tarshyne	-	1	-	1	0.6
Others	1	3	3	7	4
Total	63	99	11	173	100

A maximum of two different serotypes were isolated from individual slaughter swine including isolates from carcass swabs (Table 11). Seventeen pigs were found to have two different serovars in different samples.

Table 11: Multiple serotypes isolated from different samples of individual slaughter swine in Addis Ababa abattoir

CC	MLN	CS	No. of animals
S. Hadar	*S.* Saintpaul	-	6
S. Hadar	*S.* Typhimurium	-	5
-	*S.* Typhimurium	*S.* Eastbourne	3
S. Typhimurium var. Copenhagen	*S.* Saintpaul	-	1
S. Typhimurium var. Copenhagen	*S.* Typhimurium var. Copenhagen	*S.* Hadar	1
-	*S.* Newport	*S.* Havana	1
S. Anatum	*S.* Newport	*S.* Anatum	1
S. Hadar	*S.* Amager	-	1
	S. Typhimurium var. Copenhagen		
S. Eastbourne	Copenhagen	-	1
S. Newport	*S.* Kentucky	-	1
S. Kentucky	*S.* Eastbourne	-	1
S. Muenchen	*S.* Typhimurium	-	1

4.3. Antimicrobial resistance

All of the isolates obtained during the study (n=173) were tested for resistance to 24 different antimicrobials. Fifty-seven (32.9%) of the isolates were found resistant to one or more antimicrobials. In addition, all of the resistant isolates were multidrug resistant (MDR).

Out of the 17 serotypes recovered during the study 4 (23.53%) showed resistance to antimicrobials. The resistant isolates belong to the serovars *S.* Hadar, *S.* Kentucky, *S.* Typhimurium, and *S.* Braenderup. None of the isolates belonging to serovars *S.* Eastbourne, *S.* Saintpaul, *S.* Typhimurium var. Copenhagen, *S.* Enteritidis, *S.* Newport, *S.* Anatum, *S.* Amager,

S. Infantis, *S.* Kottbus, *S.* Muenchen, *S.* Havana, *S.* Meleagridis, and *S.* Tarshyne were resistant to any of the antimicrobials tested.

Antimicrobial resistance was found markedly associated with serovar ($P<0.001$). *Salmonella* Hadar alone represented 80.7 % (46/57) resistant isolates while *S.* Hadar with *S.* Kentucky represented 89.5% (51/57) of resistant isolates. *Salmonella* Hadar, the dominant isolate, was 97.9% (46/47) resistant to 2 or 3 antimicrobials. Its resistance, however, was restricted to nitrofurantoin, streptomycin and tetracycline. All isolates of *S.* Kentucky were multidrug resistant for 4 up to 9 antimicrobials, while 2 out of 9 *S.* Typhimurium isolates were multidrug resistant to 10 antimicrobials. *Salmonella* Braenderup was resistant to 6 antimicrobials. The other resistant serotypes were resistant to both streptomycin and tetracycline or for nitrofurantoin, streptomycin and tetracycline (Table 12). Prevalence of resistance to each antimicrobial varies with serovar (Table 13). *Salmonella* Hadar, for example was 93.6% resistant to nitrofurantoin while *S.* Kentucky was fully susceptible. On the other hand *S.* Kentucky was 100% resistant to nalidixic acid while *S.* Hadar was fully susceptible.

Table 12: Antimicrobial resistance patterns of serovars of *Salmonella* isolated from slaughtered swine in Addis Ababa

Serovar	No. of isolates		Antimicrobial resistance pattern
	Tested	Resistant	
S. Hadar	47	46	NitStrTet* (44)
			StrTet (2)
S. Eastbourne	39	-	
S. Saintpaul	37	-	
S. Typhimurium	9	2	AmpAmeChlCipFenNalSptStrSulTet (2)
S. Typhimurium var Copenhagen	6	-	
S. Kentucky	5	5	AmpAmeCipNalStrSulTet (1)
			AmpAmeCetCipNalStrSulTet (1)
			AmpAmeCipGenNalSptStrSulTet (2)
			AmpAmeCipNal (1)
S. Enteritidis	4	-	
S. Newport	4	-	
S. Anatum	3	-	
S. Amager	2	-	
S. Infantis	2	-	
S. Kottbus	2	-	
S. Muenchen	2	-	
S. Braenderup	1	1	AmpSptStrSulSxtTmp
S. Havana	1	-	
S. Meleagridis	1	-	
S. Tarshyne	1	-	
Others	7	3	NitStrTet (3)
Total	173	57	

*for abbreviations refer Table 2

Table 13: Antimicrobial resistance of *Salmonella* serovars isolated from slaughter swine in Addis Ababa

Serovar	Total isolates	Antimicrobial resistant isolates																							
		amk*	amp	amc	apr	crb	cef	cfo	ctf	fox	chl	cip	fen	gen	kan	nal	neo	nit	spt	str	sul	sxt	tet	tob	tmp
S. Hadar	47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	44	-	46	-	-	46	-	-
S. Eastbourne	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Saintpaul	37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium	9	-	2	2	-	-	-	-	-	-	2	2	2	-	-	2	-	-	2	2	2	-	2	-	-
S. Typhimurium var Copenhagen	6																								
S. Kentucky	4		-	3	-	-	1	-	-	-	2	-	-	2	-	3	-	-	2	4	4	-	4	-	-
S. Enteritidis	4																								
S. Newport	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Anatum	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Amager	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Infantis	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Kottbus	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Muenchen	2																								
S. Braenderup	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1	-	-	1
S. Havana	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Meleagridis	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Tarshyne	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Others	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	3	-	-	3	-	-
All	173	-	8	7	-	-	1	-	-	-	2	7	2	2	-	7	-	47	5	56	7	1	55	-	1

*For abbreviations refer to Table 2

Among the antimicrobials against which the *Salmonella* isolates were tested, most frequent resistance was encountered for streptomycin. Fifty-six (32.4%) isolates were resistant to streptomycin, while 55 (31.8 %) and 47 (27.2 %) demonstrated resistance to tetracycline and nitrofurantoin respectively. All resistant serotypes (Hadar, Typhimurium, Kentucky and Braenderup) showed resistant to streptomycin whereas 3 serotypes (Hadar, Typhimurium and Kentucky) were resistant to tetracycline and only *S.* Hadar demonstrated resistance to nitrofurantoin (Table 12). No resistance was detected for amikacin, apramycin, carbadox, ceftriaxone, ceftiofur, cefoxitin, kanamycin, neomycin and tobramycin.

Resistance was rare for chloramphenicol (1.2%), florfenicol (1.2%), gentamicin (1.2%), trimethoprim (0.6%), sulfamethoxazole trimethoprim (0.6%), and cephalothin (0.6%) (Table 14). Only one isolate (*S.* Braenderup) was resistant to trimethoprim and sulfamethoxazole/trimethoprim whereas an isolate from serovar *S.* Kentucky was the only one which exhibited resistance to cephalothin. Only two isolates of *S.* Kentucky, from single animal, were the only ones found resistant for gentamicin. Resistance against chloramphenicol was detected only in two isolates belonging to the serotype *S.* Typhimurium; these same isolates were also resistant to florfenicol.

Table 14: Resistance of *Salmonella* isolates (n = 173) to antimicrobials from slaughter swine in Addis Ababa

Antimicrobial	Resistant isolates	
	Number	%
Streptomycin	56	32.4
Tetracycline	55	31.8
Nitrofurantoin	47	27.2
Ampicillin	8	4.6
Amoxicillin/clavulanic acid	7	4
Ciprofloxacin	7	4
Nalidixic acid	7	4
Sulfisoxazole	7	4
Spectinomycin	5	2.9
Chloramphenicol	2	1.2
Florfenicol	2	1.2
Gentamicin	2	1.2
Cephalothin	1	0.6
Sulfamethoxazole-trimethoprim	1	0.6
Trimethoprim	1	0.6

Eight antimicrobial resistance patterns were identified (Table 15). The resistance ranges from resistance to 2 (StrTet) antimicrobials in serovar *S.* Hadar to 10 antimicrobials (AmpAmeChlCipFenNalSptStrSulTet) in serovar Typhimurium. The most frequently encountered resistance pattern was combined resistance for nitrofurantoin, streptomycin and tetracycline (NitStrTet). This pattern alone represented 47 (82.5 %) of 57 resistant isolates. Without considering streptomycin, tetracycline and nitrofurantoin, only 4.6% (8/173) of the isolates were resistant to one or more antimicrobials. Resistance for relatively large number of antimicrobials was registered for *S.* Typhimurium, *S.* Kentucky and *S.* Braenderup. *S.*

Typhimurium was resistant to 10 antimicrobials while *S.* Kentucky and *S.* Braenderup were resistant to up to 9 and 6 antimicrobials respectively.

Table 15: *Salmonella* isolates (n=173) obtained from slaughtered swine grouped by resistance pattern

Resistance pattern	Number and percentage of isolates	
	n	%
None	116	67.1
StrTet*	2	1.2
NitStrTet	47	27.2
AmpAmeCipNal	1	0.6
AmpSptStrSulSxtTmp	1	0.6
AmpAmeCipNalStrSulTet	1	0.6
AmpAmeCefCipNalStrSulTet	1	0.6
AmpAmeCipGenNalSptStrSulTet	2	1.2
AmpAmeChlCipFenNalSptStrSulTet	2	1.2
Total	173	100

*for abbreviations refer Table 5

Without considering differences in proportion of serovars in different samples, isolates from MLN were less resistant (OR=0.35, 95% CI [0.18-0.70]) compared to isolates from CC. Twenty three isolates (23.2%) out of 99 from MLN were resistant to one or more antimicrobials while 29 (46.0%) of 63 and 5 (45.5%) of 11 from CC and CS respectively were resistant to one or more antimicrobials (Table 16).

4.4. Phagetypes

Only isolates belonging to serovar Enteritidis were phagetyped. Among 4 *S.* Enteritidis isolates phagetyped one belonged to phagetype 911 and the remaining were atypical.

Table 16: Distribution of antimicrobial resistance of *Salmonella* isolates by source

Sample type	No. Examined	Serovar	Tested	Resistant (%)
Cecal content	278	*S.* Hadar	26	26 (100)
		S. Eastbourne	15	-
		S. Saintpaul	10	-
		S. Typhimurium var. Copenhagen	2	-
		S. Kentucky	2	2 (100)
		S. Newport	1	-
		S. Anatum	2	-
		S. Infantis	2	-
		S. Muenchen	1	-
		S. Meleagridis	1	-
		Others	1	1 (100)
Mesenteric lymph nodes	278	*S.* Hadar	19	18 (94.7)
		S. Eastbourne	21	-
		S. Saintpaul	27	-
		S. Typhimurium	9	2 (22.2)
		S. Typhimurium var. Copenhagen	4	-
		S. Kentucky	2	2 (100)
		S. Enteritidis	4	-
		S. Newport	3	-
		S. Amager	2	-
		S. Kottbus	2	-
		S. Muenchen	1	-
		S. Braenderup	1	1 (100)
		S. Tarshyne	1	-
		Others	3	-
Carcass swab	277	*S.* Hadar	2	2 (100)
		S. Eastbourne	3	-
		S. Kentucky	1	1 (100)
		S. Anatum	1	-
		S. Havana	3	-
		Others	3	2 (66.7)

5. DISCUSSION

5.1. *Salmonella* prevalence

In the present study, high level of *Salmonella* prevalence was observed; 43.2% of the swine carried *Salmonella* in their cecal content and/or mesenteric lymph nodes. Such high level of prevalence was also observed in 41% of swine at slaughterhouse in North Carolina, USA from cecal or lymph node samples (Gebreyes *et al.*, 2004a). However, our observation was much higher compared to a 28% finding in Belgium (Botteldoorn *et al.*, 2003). The observed high prevalence might be due to lack of awareness of the status of *Salmonella* in swine and the absence of control programs and unhygienic situations in farms of origin.

A 22.7% cecal content prevalence found in this study was in agreement with 19% prevalence in colon content reported from Belgian commercial slaughterhouses (Botteldoorn *et al.*, 2003), 18.5% isolation rate from cecal content of swine held for less than 24 h in lairage (Morgan *et al.*, 1987) and 24.6% prevalence of faecal samples in finishing swine herds in North Carolina, USA (Davies *et al.*, 1997). It was also consistent with 25% prevalence reported from Great Britain (Defra, 2003). However, it is higher than 13.6% prevalence of cecal content of abattoir necropsied swine in the USA (Hurd *et al.*, 2002) and 7.9% fecal prevalence in porcine finishing units in Quebec, Canada (Letellier *et al.*, 1999).

Isolation of *Salmonella* from 35.6% of mesenteric lymph nodes in our study was much higher compared to the report of recovery of *Salmonella* in 1 of 16 (6.25%) pooled samples of 10 mesenteric lymph nodes in Addis Ababa abattoir by Pegram and associates (1981). It is also higher than 21% isolation reported from Belgium (Botteldoorn *et al.*, 2003). Presence of salmonellae in the mesenteric lymph nodes represents a successful invasion of the host (Samuel *et al.*, 1980).

Carcass swab prevalence of 4.0% of this study is in agreement with 3.8% prevalence of *Salmonella* in swine carcass swabs reported from 7 slaughterhouses from 5 European countries (Hald *et al.*, 2003) and 4.2% report from carcass swabs in the UK (Defra, 2003). However, it

is much lower than 37% prevalence reported from 5 Belgian commercial slaughterhouses (Botteldoorn *et al*., 2003) and 9.3% carcass contamination reported by Morgan and colleagues (1987) for swine held for less than 24 hours in lairage; and higher than a no *Salmonella* detection rate in 5 swine slaughterhouses in Sweden, where there has been *Salmonella* control practice in meat- and egg-producing animals for over the past 40 years (Thorberg and Engvall, 2001). Lower prevalence of carcass swab despite high contamination level of cecal content and mesenteric lymph nodes might be due to small number of animals slaughtered per slaughter day with involvement of relatively large number of workers which would make implementation of hygienic procedures easy.

Scalding water samples were collected on 16 slaughter days with sterile vials during slaughtering operation. From these 16 samples no *Salmonella* was recovered. Our finding was in agreement with the finding from 5 Belgian slaughterhouses (Botteldoorn *et al*., 2003) where no *Salmonella* was detected from scalding water samples. However, it is lower than 2.2% contamination with *Salmonella* of water from scalding tanks of 7 slaughterhouses in four European countries (Hald *et al*., 2003). The absence of *Salmonella* in scalding water in this study seems to be due to high scalding water temperature (65 to 76.6°C) recorded during slaughter activities. In the United States for example, operating parameters for scald operations range from 57.7°C – 61°C for three to eight minutes (Dickson *et al*., 2005).

Comparison of occurrence of salmonellae in CC and MLN samples demonstrated higher ($P<0.001$) MLN contamination (35.6%) than cecal content (22.7%). This result was in agreement with a 21 and 19% report for these two samples respectively (Botteldoorn *et al*., 2003) and a finding of 2.8% for fecal samples and 0.5% for MLN of sheep and goats at abattoir in Debre Zeit, Ethiopia (Woldemariam *et al*., 2005). Alemayehu and associates (2005) also reported *Salmonella* from relatively larger proportion of MLN (1.2%) than feces (0.62%) of cattle at abattoir in Debre Zeit. Result of this study, however, was different from a higher prevalence (39%) for cecal sample than MLN (21%) reported from North Carolina, USA (Gebreyes *et al*., 2004a). It is worth mentioning that the later study used resected cecum instead of cecal content used in our study. On the other hand Molla and associates (2004a) isolated

Salmonella from the two samples in equal proportions (15.1% fecal samples and 15.9% MLN) from apparently healthy slaughtered camels.

Swine, which carried *Salmonella* in their cecal content, were found having more than 6 fold higher probability (OR = 6.7) of their carcasses being contaminated with *Salmonella* than those which were negative for their cecal content. In addition, carcass contamination with *Salmonella* was not observed on slaughter days which were culture negative for CC and MLN. These findings indicate the importance of *Salmonella* carried by swine, especially in the gut, to the slaughter line on carcass contamination. This result is in agreement with the findings of Morgan *et al.* (1987) who found that the source of carcass contamination in swine with *Salmonella* was primarily intestinal *Salmonella* infections. According to Berends *et al.* (1997), live animals that carry *Salmonella* are 3 to 4 times more likely to end up as a positive carcass than *Salmonella*-free animals. Though high in prevalence (35.6 %) there was no statistical association between mesenteric lymph node prevalence and carcass contamination. The reason might be, as solid enclosed tissue, lymph nodes do not have a high chance of contaminating butcher's hands and carcass.

In addition to the statistically significant association of CS contamination with CC *Salmonella* status, similarity of serotypes isolated from CC and/or MLN of the same animal, or animals slaughtered on the same slaughter day, supplement the finding that *Salmonella* from slaughter swine were important source of carcass contamination. Eight of the serotypes isolated from carcass swab samples were either recovered from CC and/or MLN samples of the same animals or other animals slaughtered that day (Table 8). Moreover, antimicrobial resistance patterns of similar serovars recovered from other samples of the same animal or animals slaughtered on the same date were also similar with the exception of one isolate (Table 9). Significance of *Salmonella* in gut contents and lymph nodes as a major source of contamination of the carcass and edible offal after slaughter was stated earlier (Samuel *et al.* 1980).

High prevalence of *Salmonella* was observed in slaughter swine in this study. This high prevalence of *Salmonella* observed in our study might partly be due to stress of transportation (Isaacson *et al.*, 1999), a natural infection during transport (Schwartz, 1993; Hurd *et al.*, 2002; Vieira-Pinto *et al.*, 2006a) and lairage (Morgan *et al.*, 1987; Hurd *et al.*, 2002), stress of

commingling (Schwartz, 1993) and uncomfortable situation in lairage or it might be due to conditions which favor *Salmonella* transmission and maintenance at the farms of origin. Swine with *Salmonella* can shed organisms in feces, which can then serve as a source of contamination to other swine, the environment, mechanical devices used in slaughter or processing, and ultimately pork products (Morgan *et al*., 1987; Berends *et al*., 1997).

High *Salmonella* prevalence in swine is important because of the potential hazard they pose to human health (Schwartz, 1993). The United States Department of Agriculture has identified *Salmonella* enterica as the most important of the known foodborne pathogens and pork considered an important source (Davies *et al*., 1997).

5.2. Serotypes

One hundred and sixty six of the 173 isolates recovered during this study were identified as specific serovars. These serovar-identified isolates belonged to 17 serotypes. Comparison of type and relative frequency of serovars with previous works conducted on swine in Ethiopia was not possible, as there were no works conducted on *Salmonella* in swine except that of Pegram *et al* (1981) which was carried out on 16 pooled mesenteric lymph node samples at Addis Ababa abattoir.

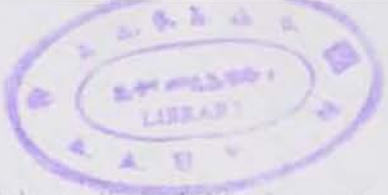
In our study the most frequent serotype isolated was S. Hadar (27.2%). This serovar was reported in Ethiopia earlier from goats (Woldemariam *et al*., 2005), camels (Molla *et al*., 2004a), poultry (Molla and Mesfin, 2003), chicken (Zewdu, 2004) and mutton (Zewdu, 2004) at 4.3, 2.6, 2.2, 6.1 and 2.0% relative serovar prevalence respectively. However, the proportion with which this serovar was reported from these animals was not as high as what was observed in our study. Moreover, this serovar was not either among the most frequently isolated serovars from swine elsewhere (Morgan *et al*., 1987; Gupta and Verma, 1990; Letellier *et al*., 1999; Anonymous, 2000; Gebreyes *et al*., 2000; Funk *et al*., 2000; van Duijkeren *et al*., 2002; Defra, 2003; Rowe *et al*., 2003; Rajic *et al*., 2005). However, *Salmonella* Hadar was one of the most common laboratory confirmed *Salmonella* serotypes isolated from people elsewhere (Anonymous, 2000; Defra, 2003; Bangtrakulnonth *et al*., 2004). The serovar's short progression

has also been noted especially in 1994 and 1995 in many European countries (Uyttendaele, 1998). Considering the serotype's high prevalence in swine in Ethiopia and its ability to infect humans, our finding implies the potential role swine could play in the zoonoses of salmonellosis in Ethiopia.

The second most frequent serovar isolated in our study was *Salmonella* Eastbourne. This serovar was reported from a previous work in Ethiopia in camels (Pegram *et al.*, 1981). Contrary to its dominance in our isolates it is not a serotype frequently reported from swine in other countries, as was *S.* Hadar (Morgan *et al.*, 1987; Gupta and Verma, 1990; Letellier *et al.*, 1999; Anonymous, 2000; Gebreyes *et al.*, 2000; Funk *et al.*, 2000; van Duijkeren *et al.*, 2002; Defra, 2003; Rowe *et al.*, 2003; Raine *et al.*, 2005).

Salmonella Saintpaul, the third most frequently isolated serovar (21.4%) in this study, was the only serotype reported from slaughter swine in Ethiopia earlier. It was isolated from pooled sample of mesenteric lymph nodes in Addis Ababa (Pegram *et al.*, 1981). This serovar was also reported in high relative proportions from camels (Molla *et al.*, 2004a) and minced beef samples in Ethiopia by different workers (Nyaleti *et al.*, 2000; Ejeta *et al.*, 2004; Zewdu, 2004). The predominance of this serotype in this study is again in contradiction to reports from different countries in swine (Morgan *et al.*, 1987; Gupta and Verma, 1990; Letellier *et al.*, 1999; Anonymous, 2000; Gebreyes *et al.*, 2000; Funk *et al.*, 2000; van Duijkeren *et al.*, 2002; Defra, 2003; Rowe *et al.*, 2003; Raine *et al.*, 2005). *Salmonella* Saintpaul was among serovars isolated from humans (Anonymous, 2000). The detection of this serovar at high prevalence in the present study and the fact that it was the first and only serotype reported from swine in Ethiopia may suggest the serovars dominance in swine populations of the country across time.

Salmonella Typhimurium with variety Copenhagen made 8.7% of the total isolates of *Salmonella*. Five point two percent was *S.* Typhimurium while 3.5% was *S.* Typhimurium var. Copenhagen. *Salmonella* Typhimurium was among the most frequently reported serovars from animals in Ethiopia. It was reported from goats (Molla, 2004; Woldemariam *et al.*, 2005), camels (Molla *et al.*, 2004a), poultry (Molla and Mesfin, 2003; Tibbituka *et al.*, 2003), cattle (Wondimagegnhu *et al.*, 2003), sheep (Molla, 2004) and goats (Woldemariam *et al.*, 2005).



Though there was virtually no study conducted on *Salmonella* in Ethiopian swine to compare with, the relative prevalence of *Salmonella* Typhimurium recorded in this study was by far lower than recorded for swine elsewhere; it was the most common serovar isolated from swine at slaughter in the UK and Denmark (Anonymous, 2000; Defra, 2003). It also was the most prevalent serotype recorded in swine from different countries (Anonymous, 2000; Gebreyes *et al.*, 2000; van Duijkeren *et al.*, 2002; Defra, 2003; Rajic *et al.*, 2005) and pork (Anonymous, 2000). Our result was, however, comparable to a 2.2% prevalence reported from Australia (Morgan *et al.*, 1987).

Isolation of *S.* Typhimurium may imply its animal health significance in addition to its public health importance, as it is one of the causes of salmonellosis in swine (*Schwartz, 1993; Roof et al., 1993*). It was the most frequent serotype isolated from outbreaks of clinical disease in swine herds in Denmark in 1999 (Anonymous, 2000). *Salmonella* Typhimurium is among the most prevalent serovars in Europe and America. It was for instance the second most important serotype isolated from humans in Denmark (Anonymous, 2000) and UK (Defra, 2003) while it was the most common serotype isolated from humans in USA in recent years (CDC, 2003). *Salmonella* Typhimurium was the most prevalent serotype in humans during the period from 1984 to 2001 in The Netherlands (van Duijkeren *et al.*, 2002). Pork was incriminated as source of *S.* Typhimurium infections in humans in many instances (Anonymous, 2000; Defra, 2003). The presence of this serovar in MLN and CC of swine indicate the potential of pork contamination from these sources.

Salmonella Enteritidis comprised 2.5% of the total isolates in this study. The serovar was reported earlier in Ethiopian cattle (Alemayehu *et al.*, 2003), camels (Molla *et al.*, 2004a), poultry (Molomo, 1998), and sheep (Molla, 2004). Our result is higher than 0.6% prevalence of this serovar in Danish slaughter swine (Anonymous, 2000) and about 0.4% prevalence in slaughter swine in UK (Defra, 2003).

Salmonella Enteritidis is the most common serovar of *Salmonella* isolated from humans in different countries (Anonymous, 2000; van Duijkeren *et al.*, 2002; Defra, 2003; Bangtrakulnonth *et al.*, 2004). The number of serovar Enteritidis isolates has been increasing in The Netherlands and since 1993 it has been the most frequently isolated serovar from humans

(van Duijkeren *et al.*, 2002). Pork was incriminated as a source of *S.* Enteritidis outbreak in humans in Denmark in 1999 (Anonymous, 2000). The presence of this well-known zoonotic serovar in relatively higher prevalence in swine in Ethiopia indicates the role that swine could play in food borne salmonellosis in the country. *Salmonella* Enteritidis is also occasionally incriminated in disease outbreaks in swine (Schwartz, 1993).

Salmonella Newport comprised 2.3 % of isolates identified in our study. This serovar was the most common isolate recovered from food samples of animal origin and stool samples from supermarket personnel in a recent study in Ethiopia (Zewdu, 2004). The serotype is among the 3 most common serotypes isolated from humans in the USA and it is on the increase (CDC, 2002). It was among the top 10 laboratory confirmed *Salmonella* serotypes isolated from people in 2001 in UK. This serovar was also among the top 10 *Salmonella* Serotypes in swine incidents in 2001 in UK with relative occurrence of 1.4% (Defra, 2003), which is comparable to our finding. *Salmonella* Newport was among the three most frequently isolated serovars from pigs in India (Gupta and Verma, 1990).

Salmonella Anatum was 1.7% of the serovars identified in this study. It was reported earlier in Ethiopian cattle (Nyeleti *et al.*, 2000), goats (Woldemariam *et al.*, 2005), camel (Molla *et al.*, 2004a), chicken meat (Tibaijuka *et al.*, 2005; Molla and Mesfin, 2006; Zewdu, 2004), minced beef (Nyeleti *et al.*, 2000; Ejetat *et al.*, 2004; Zewdu, 2004), mutton (Ejetat *et al.*, 2004), and abattoir personnel (Nyeleti *et al.*, 2000). It is one of the most frequently isolated serovars elsewhere from swine (Morgan *et al.*, 1987; Gupta and Verma, 1990; Letellier *et al.*, 1999; Funk *et al.*, 2000). *Salmonella* Anatum has consistently been one of the most important causes of salmonellosis in humans in Thailand (Bangtrakulnonth *et al.*, 2004).

This is, as to our knowledge, the first report of *S.* Amager and *S.* Tarshyne in this country. They were isolated at lower frequency (1.2% for *S.* Amager and 0.6% for *S.* Tarshyne) and they are rarely isolated serotypes elsewhere too. They are not reported in many works conducted in swine in different countries (Morgan *et al.*, 1987; Letellier *et al.*, 1999; Funk *et al.*, 2000; Gebreyes *et al.*, 2000; van Duijkeren *et al.*, 2002; Rowe *et al.*, 2003).

Salmonella Infantis, *S.* Kottbus and *S.* Muenchen were each isolated at 1.2 % relative prevalence. *Salmonella* Infantis was isolated from sheep and goats (Woldemariam *et al.*, 2005), camels (Molla *et al.*, 2004a), poultry (Molla and Mesfin, 2003), minced beef and mutton (Zewdu, 2004), and pork (Ejetat *et al.*, 2004; Zewdu, 2004) earlier in Ethiopia. Though *S.* Infantis was recovered from pork at 33.3% prevalence previously in Ethiopia (Ejetat *et al.*, 2004), it was not among the dominant serotypes isolated in our study. The serovar was reported from swine in many countries at varying frequency (Letellier *et al.*, 1999; Gebreyes *et al.*, 2000; Anonymous, 2000; Rowe *et al.*, 2003; Rajic *et al.*, 2005). It is one of the top ten laboratory confirmed *Salmonella* serotypes isolated from people (Anonymous, 2000; Defra, 2003).

Salmonella Kottbus was reported from poultry (Molla and Mesfin, 2003), camels (Molla *et al.*, 2004a), pork (Zewdu, 2004) and sheep and goats (Woldemariam *et al.*, 2005) in Ethiopia. However, it seems that it is less frequently isolated serotype from swine, as it was not reported in studies conducted in different countries (Letellier *et al.*, 1999; Gebreyes *et al.*, 2000; Anonymous, 2000; Rowe *et al.*, 2003; Rajic *et al.*, 2005).

The remaining serotypes *S.* Braenderup, *S.* Havana and *S.* Meleagridis, each constituted 0.6 % of the isolates. *Salmonella* Braenderup was one of the most frequently isolated serotypes from animals and animal products in Ethiopia. It was reported from sheep and goats (Woldemariam *et al.*, 2005), camels (Molla *et al.*, 2004a), poultry (Molla and Mesfin, 2003; Tibbayuka *et al.*, 2003), chicken (Zewdu, 2004), minced beef and mutton (Ejetat *et al.*, 2004), and pork (Ejeta *et al.*, 2004). It is one of the top 10 laboratory confirmed serotypes isolated from people in E. in 2001 (Defra, 2003) and one of the serotypes isolated from humans in Denmark in 1999 (Anonymous, 2000). *Salmonella* Havana was reported from European camels earlier (Molla *et al.*, 2004a). It was also among the most common isolates from swine in Australia (Morgan *et al.*, 1987). *Salmonella* Meleagridis, however, was reported only from stool of abattoir personnel in Ethiopia (Nyeleti *et al.*, 2000).

There is a marked difference in serovars causing infections in swine in Ethiopia and in other countries. This might be related to the available reservoirs for persistence and spread of *Salmonella* infection (Baneryakhonoth *et al.*, 2004). However, as Santpaul, the only serovar

reported before 25 years from swine was found one of the dominant serovars in our study. It might be worth mentioning at this point that researchers who analyzed a very large data reported change in serotype distribution over a study period. These researchers also demonstrated considerable difference in the distribution of sero- and phage types between chickens, swine, and cattle (van Duijkeren *et al.*, 2002). Recovery of 17 serotypes from swine in our study demonstrated the diversity of serotypes of *Salmonella* infecting swine in Ethiopia.

5.3. Antimicrobial resistance

All of the isolates recovered in the study were tested against a panel of 24 antimicrobials for detection of presence of resistance. Out of 173 isolates tested, 57 (32.9%) were found resistant to two or more antimicrobials. Our result compares favorably with 31.8% and 32.7% overall resistance reported for isolates from sheep and goats and food samples by Molla (2004) and Zewdu (2004) respectively in Ethiopia. Our result, however, was much lower than 57.4%, 93.3%, 93.2%, 79%, 63.7%, 44.8% prevalence of antimicrobial resistance for one or more drugs in isolates from chicken carcasses and giblets (Tibaijuka *et al.*, 2002), diarrhoeal out-patients (Mache *et al.*, 1997), diarrhoeal pediatric out-patients (Mache, 2002), human beings (Gedebou and Tassew, 1981), chicken carcasses and giblets (Molla *et al.*, 2003b) and camels (Molla *et al.*, 2004b) respectively in Ethiopia. The resistance status observed in this study was better than 85.1% resistance reported for one or more antimicrobial in *Salmonella* isolates from pigs in North Carolina (Gebreyes *et al.*, 2000). The difference observed in antimicrobial resistance level of our isolates compared with the above mentioned works might be due to difference in serotypes of isolates recovered in respective studies or it might be due to difference in frequency of use of antimicrobials in different species. Comparison of resistance of our isolates with isolates from swine and analysis of trend of resistance among *Salmonella* isolates from swine in Ethiopia was not possible because of absence of works on salmonellae of swine in the country.

All of the resistant isolates were resistant to at least 2 antimicrobials (multidrug resistant) this was in agreement with a 100% multidrug resistance reported by Molla (2004) among isolates from sheep and goats. Multidrug resistance was higher in our isolates compared to its 54.8%

and 52.5% prevalence in isolates from chicken carcasses and giblets (Tibaijuka *et al.*, 2002; Molla *et al.*, 2003b), 71.9% prevalence in isolates from food samples (Zewdu, 2004), and 33.6 % prevalence in isolates from camels (Molla *et al.*, 2004b).

Forty-six (97.9%) out of 47 *S.* Hadar isolates were multidrug resistant. Resistance of this serovar for antimicrobials was reported from camels (Molla *et al.*, 2004b) and meat samples (Zewdu, 2004) earlier in Ethiopia. This serotype was resistant to streptomycin, tetracycline and nitrofurantoin. Single or combined resistance of this serotype to streptomycin and tetracycline was observed in isolates from meat samples (Zewdu, 2004) and a combined resistance for streptomycin and tetracycline in isolates from camels (Molla *et al.*, 2004b). However, the resistant isolates of this serotype in our study were all multidrug resistant and moreover they were additionally resistant to nitrofurantoin.

Salmonella Kentucky isolated in this study were all multidurg resistant for 4 up to 9 serotypes. High level of multidrug resistance, consistent to our result, was observed on isolates of this serotype from meat samples in Ethiopia earlier (Zewdu, 2004). In addition to the serovar's 100% multidug resistance, the resistance pattern of the isolates from this study and Zewdu's study were more or less the same. *Salmonella* Kentucky was also among most resistant serotypes isolated from feedlot cattle in USA (Dargatz *et al.*, 2002).

Only 2 of 9 (22.2%) *S.* Typhimurium isolates were resistant to antimicrobials; however, these isolates were the only ones, which demonstrated resistance for 10 antimicrobials in this study. Very high level of multiple drug resistance of this serotype was registered in camels (83.3%) (Molla *et al.*, 2004b), sheep and goats (62.5%) (Molla, 2004) and chicken carcasses and giblets (33.3%) (Molla *et al.*, 2003b) in Ethiopia. High level of multiple drug resistance was also reported on *S.* Typhimurium isolates from swine (Gebreyes *et al.*, 2000; Gebreyes *et al* 2004) and feedlot cattle (Dargatz *et al.*, 2002) in USA. Our isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, in addition to other antimicrobials. The multidrug resistant *S.* Typhimurium DT104 is known to show resistance to these antimicrobials (Mølbak *et al.*, 1999). The observation of this resistance pattern may indicate existence of the strain in Ethiopia. Pigs could play important role in zoonosis of multidrug resistant *S.* Typhimurium (Mølbak *et al.*, 1999).

The single isolate of *S.* Braenderup obtained from the study was found to be multidrug resistant to 6 antimicrobials. This result agrees with previous reports of high prevalence of multiple drug resistance among isolates of *S.* Braenderup in this country. Such high resistance to antimicrobials with a more or less similar pattern was reported for this serotype isolated from camels (Molla *et al.*, 2004b), chicken carcasses and giblets (Tibaijuka *et al.*, 2002; Molla *et al.*, 2003b) in Ethiopia.

In other studies *S.* Typhimurium var. Copenhagen, obtained from different sources was frequently identified to be multidrug resistant (Gebreyes *et al.*, 2000; Molla *et al.*, 2003b; Gebreyes *et al* 2004). Again unlike our findings, resistance was registered for *S.* Saintpaul isolates obtained from chicken meat and giblets in Ethiopia (Tibaijuka *et al.*, 2002). Various workers reported resistance to antimicrobials in *S.* Anatum isolated from different sources in different countries (Morgan *et al.*, 1987; Tibaijuka *et al.*, 2002; Dargatz *et al.*, 2002; Molla *et al.*, 2003b). Resistance was also reported in *S.* Infantis isolated from slaughter swine in Australia (Morgan *et al.*, 1987). Antimicrobial resistance of *S.* Havana isolated from slaughter swine was reported from Australia (Morgan *et al.*, 1987) but isolates obtained from camels in Ethiopia were all reported sensitive (Molla *et al.*, 2004b). As it was the case in our study *S.* Muenchen isolated earlier in Ethiopia from camel was also sensitive for all antimicrobials tested (Molla *et al.*, 2004b).

Streptomycin was the least effective antimicrobial. Out of 173 total isolates 56 (32.4%) were resistant to streptomycin. Compared to the 57 resistant isolates identified in the study 98.2% (56/57) of the resistant isolates were resistant to streptomycin. Various workers have reported comparable high level of resistance of salmonellae to streptomycin from different sources in Ethiopia (Tibaijuka *et al.*, 2002; Molla *et al.*, 2003b; Molla, 2004, Zewdu, 2004; Molla *et al.*, 2004b).

Thirty one point two percent (55/173) of *Salmonella* isolates recovered during this study were tetracycline resistant. Varying level of resistance of salmonellae has been reported from Ethiopia from different sources. Lower resistance to tetracycline, compared to this study, was reported by Molla (2004) on isolates from sheep and goats (13.6%), Zewdu (2004) on isolates from food items (15.3%) and Molla *et al* (2004b) on isolates from camels (12.9 %). On the

other hand higher resistance, compared to our result, was reported frequently, especially on human isolates in Ethiopia. Seventy one point one percent of isolates from diarrhoeal outpatients in Addis Ababa were resistant of tetracycline (Mache *et al.*, 1997) while 59.3% isolates from diarrhoeal pediatric outpatients in Jimma were resistant (Mache, 2002). However, susceptibility of most isolates from humans has been reported (Gedebou and Tassew, 1981). Much higher level of resistance has also been reported on isolates from animals and animal products in Ethiopia and elsewhere. Molla and associates (2003b) reported a 41.2% resistance among isolates of *Salmonella* from chicken carcasses and giblets in Ethiopia. While as high as 84.2% and 64.5% resistance to tetracycline among *Salmonella* isolates was reported from North Carolina (Gebreyes *et al.*, 2000) and on isolates from pork carcasses in Northeast Georgia (Epling and Carpenter, 1990) respectively. This result is also lower than in The Netherlands where 40 % of isolates from swine were resistant to tetracycline (van der Wolf *et al.*, 1999).

Nitrofurantoin resistance was the third frequent resistance observed in this study, 47 out of 173 (27.2%) isolates were resistant to this antimicrobial. Nitrofurantoin resistance was not found on 80 *Salmonella* isolates obtained from chicken carcasses and giblets from Debre Zeit and Addis Ababa (Molla *et al.*, 2003b).

Resistance of salmonellae for ampicillin was 4.6% and it was 4% for amoxicillin/clavulanic acid, ciprofloxacin, nalidixic acid and sulfisoxazole each. Several workers have reported a relatively high level of ampicillin resistance among *Salmonella* isolates from different sources in different countries (Epling and Carpenter, 1990; Mache *et al.*, 1997; van der Wolf *et al.*, 1999; Gebreyes *et al.*, 2000; Tibaijuka *et al.*, 2002; Mache, 2002; Molla *et al.*, 2003b; Molla *et al.*, 2004b; Molla, 2004; Zewdu, 2004; Gebreyes *et al.*, 2004b). A low level of resistance against amoxicillin/clavulanic acid (4%) was reported as compared to resistance reports for this antimicrobial by other workers i.e. Gebreyes *et al.* (2000), Molla *et al.* (2003b), and Gebreyes *et al* (2004).

Resistance against ciprofloxacin was 4%. No resistance was registered against this antimicrobial by (Molla *et al.*, 2003b; Molla *et al.*, 2004b; Gebreyes *et al.*, 2004b). Hsueh *et al* (2004) reported an overall ciprofloxacin resistance of 2.7% (18/671) of all nontyphoid *Salmonella* isolates from humans in Taiwan. The detection of 4% resistance against this

floroquinolone antibiotic signifies its implications in treatment of human patients. Pigs were reported to be sources of ciprofloxacin resistant *S.* Typhimurium to humans (Hsueh *et al.*, 2004). Resistance against this antibiotic, in the present study, was observed in serovars *S.* Kentucky and *S.* Typhimurium. Its presence especially in the well-known zoonotic serovar, *S.* Typhimurium implies the very narrow option with which health professionals would be left with in treating their patients. The fact that some of these isolates were simultaneously resistant to chloramphenicol, gentamicin and florfenicol in addition to the other quinolone nalidixic acid further complicates the situation.

All isolates, which were resistant to ciprofloxacin, were also resistant to nalidixic acid. This may show a cross resistance against the two quinolones. Earlier reports from Ethiopia, however, indicated susceptibility of all tested isolates to this antimicrobial (Molla *et al.*, 2003b; Molla *et al.*, 2004b). Public and animal health implications of resistance against nalidixic acid are the same as that to ciprofloxacin. To our knowledge this is the first report of resistance against ciprofloxacin and nalidixic acid in this country. The high prevalence to ciprofloxacin documented in our investigation may be associated with importation of contaminated products and use of fluoroquinolones in humans. Quinolones are not frequently used in veterinary medicine in Ethiopia.

Various workers reported sulfisoxazole resistance on isolates from different sources in Ethiopia (Molla *et al.*, 2003b; Molla, 2004; Zewdu, 2004). The 4% resistance of *Salmonella* isolates of this work is much lower than reports of these researchers.

The multidrug resistant isolates belonging to *S.* Typhimurium, *S.* Braenderup and *S.* Kentucky were also found resistant to spectinomycin. About 2.9 % of the total isolates were resistant to this antimicrobial. As compared to works conducted by other investigators i.e. Tibaijuka *et al.* (2002), Molla *et al.* (2003b), Zewdu (2004) and Molla *et al.* (2004b) in Ethiopia, result of the present study is much lower.

Two isolates (1.2%) were resistant to chloramphenicol. Resistance against this antibiotic was recorded earlier in Ethiopia and elsewhere (Mache *et al.*, 1997; Gebreyes *et al.*, 2000; Mache, 2002; Molla *et al.*, 2003b; Gebreyes *et al* 2004). The proportion of isolates with

chloramphenicol resistance in the present study, however, was lower. Resistance against this antibiotic was observed only in *S.* Typhimurium. A high correlation was reported between *Salmonella* Typhimurium and chloramphenicol resistance (Gebreyes *et al.*, 2004b). The two isolates, which were resistant to chloramphenicol, were resistant to 10 antimicrobials in total. Gedebou and Tassew, (1981) reported susceptibility of all tested isolates against this antibiotic in Ethiopia 24 yeas ago. Repeated detection of resistance against this antibiotic since then demonstrates increasing resistance of salmonellae with time to this commonly used antimicrobial in humans in Ethiopia.

Salmonella Typhimurium isolates, which were resistant to chloramphenicol, were the only ones, which were resistant to florfenicol too. This finding is in agreement with the work of Molla *et al.* (2003b) as resistance against this antibiotic was recorded among *S.* Typhimurium isolates from chicken carcasses and giblets.

The result we obtained, 1.2% resistance for gentamicin, agrees with 3% resistance reported from North Carolina among isolates from swine (Gebreyes *et al.*, 2000). Susceptibility of all isolates tested against gentamicin in different studies was reported (Gedebou and Tassew, 1981; Ashenafi and Gedebou, 1985; Epling and Carpenter, 1990; van der Wolf *et al.*, 1999; Molla *et al.*, 2003b; Molla *et al.*, 2004b). Both isolates resistant to gentamicin were *S.* Kentucky and were resistant to 9 antimicrobials (Table 12).

Resistance against both sulfamethoxazole/trimethoprim and trimethoprim was 0.6%. A multidrug resistant and only isolate of *S.* Braenderup was resistant for these antimicrobials. The present study is in agreement with 1.2% resistance reported by (Gebreyes *et al.*, 2000) and a no resistance report (Gedebou and Tassew, 1981; Ashenafi and Gedebou, 1985), but disagrees with much higher resistance reported for sulfamethoxazole/trimethoprim (Mache *et al.*, 1997; van der Wolf *et al.*, 1999; Mache, 2002; Tibaijuka *et al.*, 2002; Molla *et al.*, 2003b; Molla *et al.*, 2004b) and for trimethoprim (Epling and Carpenter, 1990; Tibaijuka *et al.*, 2002; Molla *et al.*, 2004b). *Salmonella* Braenderup was found associated with trimethoprim and sulfamethoxazole/trimethoprim resistance (Molla *et al.*, 2003b; Molla *et al.*, 2004b).

Differences observed in overall antimicrobial resistance between isolates of this work and works by other investigators might be due to differences in the composition of serovars identified in the studies, or it might be due to the type and frequency of antimicrobial use in different species of animals and human beings. No resistance was detected for amikacin, apramycin, carbadox, ceftriaxone, ceftiofur, cefoxitin, kanamycin, neomycin and tobramycin. In agreement with this study, susceptibility of *Salmonella* isolates to most of these antimicrobials was reported in Ethiopia (Molla *et al.*, 2003b; Molla *et al.*, 2004b). However, resistance was registered against kanamycin (Mache *et al.*, 1997; Gebreyes *et al.*, 2000) and Neomycin (Epling and Carpenter, 1990; Dargatz *et al.*, 2002).

High level of resistance, as it might be expected, was observed to frequently used antimicrobials (streptomycin, tetracycline) in animal health in the country. However, we have observed resistance to antimicrobials which are rarely or not used (ampicillin, ciprofloxacin, nalidixic acid, chloramphenicol, florfenicol, gentamicin) in veterinary medicine in Ethiopia, suggesting importance of imported contaminated items or use of antimicrobials in humanbeings or cross resistance between antimicrobials.

6. CONCLUSIONS AND RECOMMENDATIONS

There was high level of *Salmonella* infection in slaughter swine in Ethiopia. However, carcass contamination compared to the supply of *Salmonella* with slaughter swine was very low. The contamination level was low compared even to slaughterhouses in some developed countries. Low carcass contamination observed in face of high level of prevalence of *Salmonella* in cecal content and mesenteric lymph nodes indicate slaughter processes followed could reduce carcass contamination.

Supply of infected animals to the slaughterhouse was the main source of contamination of carcasses during slaughter. Swine with *Salmonella* in their cecal content were more likely to end up with contaminated carcass compared to swine which were negative.

The most frequent serotypes infecting swine in Ethiopia were different from those in other countries. This may be attributed to differences in maintenance hosts of *Salmonella* available in the swine's environment. The number of *Salmonella* serotypes (17) isolated from our study animals was high, showing diversity of serovars infecting swine in this country. About half of the serotypes isolated in this study were among the serotypes commonly isolated from human beings in different countries. Some of the serotypes are also known causes of clinical disease in swine, indicating possibility of animal health importance of *Salmonella* in swine in Ethiopia.

Multidrug resistant salmonellae were prevalent in this study, resistance was found for antibiotics that are considered first choice drugs for *Salmonella*; gentamicin, chloramphenicol, ciprofloxacin, florfenicol and nalidixic acid. There were two isolates of *S.* Typhimurium with combined resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracycline which could indicate presence of multiresistant *S.* Typhimurium DT104.

Presence of serovars which are commonly isolated from human beings coupled with their high prevalence and multidrug resistance, indicates the potential swine could play in the zoonoses of salmonellosis in Ethiopia. As this work is the first of its kind in swine in Ethiopia it would serve

as a baseline data for monitoring serotype distribution and antimicrobial susceptibility of *Salmonella* in the future.

The present study documented high prevalence of *Salmonella* in slaughter swine in Ethiopia, and the association between prevalence of *Salmonella* in slaughter swine and carcass contamination. Therefore factors that contribute to high prevalence of *Salmonella* in slaughter swine should be identified and measures taken to reduce prevalence of *Salmonella* in swine supplied to slaughter. These measures would help to reduce *Salmonella* in pork and also reduce environmental contamination. Special care should be taken during evisceration in order to reduce carcass contamination, since gut *Salmonella* status was directly related to carcass contamination.

Serovars known to infect humans and those which could induce clinical disease in swine were among the isolates of this study. There was also multiple drug resistance among these isolates. There is, therefore, a need to determine the role of these serovars in salmonellosis in swine, and monitor the serovar distribution and antimicrobial resistance patterns of *Salmonella* isolates from swine in order to trace human infections and to substantiate the risk of transfer of resistant salmonellae to humans.

7. REFERENCES

Acha, P. and Szyfres, B. (2001): Zoonoses and Communicable Diseases Common to Man and Animals; Bacterioses and mycoses. 3rd 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 Production*, **35**, 309-319.

Anderson, E. S. and Williams R. E. O. (1956): Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *Journal of Clinical Pathology*, **9**, 94-114.

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

Angulo, F.J., Nunnery J. A. and Bair H. D. (2004): Antimicrobial resistance in zoonotic enteric pathogens. *Revue Scientifique et Technique Office International des Epizooties*, **23** (2), 485-496.

Anonymous (2000): Annual Report on Zoonoses in Denmark 1999, Ministry of Food, Agriculture and Fisheries. Available at: http://www.svs.dk/

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

Bangtrakulnonth, A., Pornreongwong, S. Pulsrikarn, C., Sawanpanyalert, P., Hendriksen, R. S., Wong, D. M., Aarestrup F. M. (2004): *Salmonella* serovars from humans and other sources in Thailand, 1993–2002. *Emerging Infectious Diseases*, **10**.131-136

Berends, B. R., Van Knapen, F., Snijders J. M. and Mossel, D. A. (1997): Identification and quantification of risk factors regarding Salmonella spp. on pork carcasses. *International Journal of Food Microbiology*, **20**, 199-206.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K. and Herman (2003): *Salmonella* on pig carcasses, positive pigs and cross contamination in the slaughterhouse. *Journal of Applied Microbiology*, **95**, 891-903.

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

Brown, S. D. and Washington, J. A. (1978): Evaluation of the repliscan system for identification of Enterobacteriaceae. *Journal of Clinical Microbiology*, **8**, 695-699.

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 (2002): Preliminary FoodNet Data on the Incidence of Foodborne Illnesses- Selected Sites, United States, 2001. Morbidity and Mortality Weekly Report, **51**, 325-329.

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

CSA (2001): Central Statistical Authority, Federal Democratic Republic of Ethiopia, Central Statistical Investigatory, Statistical Abstract, 2001.

D'Aoust, J.-Y. (1989): *Salmonella*. Chapter 9. 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.

Dargatz, D.A., Fedorka-Cray, P.J., Ladely, S.R., Ferris, K.E., Green, A.L., and Headrick, M.L (2002): Antimicrobial susceptibility patterns of *Salmonella* isolates from cattle in feedlots. *Journal of American Veterinary Medical Association*, **221**,268-272.

Davies, P. R., Morrow, W. E. M., Jones, F. T., Deen, J., Fedorka-Cray, J. and Harris, I. T. (1997): Prevalence of *Salmonella* in finishing swine raised in different production systems in North Carolina, USA. *Epidemiology and Infection*, **199**, 237-244.

Davis, B. D., Dulbecco, R., Eisen, H. N., and Ginsberg, H. S. (1990): Microbiology. 4th ed. J. B. Lippincott Company. pp 576-579.

Defra (2003): Zoonoses Report, United Kingdom 2001. Available from: www.defra.gov.uk

Dickson, J.S., Hurd, H.S., and Rostagno, M.H. (2003): Salmonella in the pork production Chain. National Pork Board, Des Moines, IA. Web: http://www.porkboard.org/

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

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 Vétérinaire*, **155**, 547-551.

Epling, L.K., and Carpenter, J.A. (1990): Antibiotic resistance of Salmonella isolated from pork carcasses in Northeast Georgia. *Journal of Food Protection*, **53**(3), 253-254.

Escartin, E. F., Lozano, J. S., Rodríguez, O., Gonzales, N. M., and Torres, J. A. (1995): Incidence and level of *Salmonella* serovars in raw pork obtained from Mexican butcher shops. *Food Microbiology*. **12**, 435-439.

Ewing, W. H. (1986): Serological Identification of *Salmoenella*. In: Ewing, W. H. (ed): Edwards and Ewing's Identification of *Enterobacteriaceae*. 4thed. New York: Elsever science publishing Co., Inc. PP. 201-238.

Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, Iwen PC, *et al.* (2000): Ceftriaxone-resistant Salmonella infection acquired by a child from cattle. *New England Journal of Medicine*, 342:1242–9.

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

Funk, J. A., Davies, P. R. and Nichols M.A. (2000): The effect of fecal sample weight on detection of *Salmonella enterica* in swine feces. *Journal of Veterinary Diagnostic Investigation*, **12**, 412-418.

Gebreyes, W. A., Davies, P. R., Morrow, W. E. M., Funk, J. A. and Altier, C. (2000): Antimicrobial resistance of *Salmonella* isolates from swine. *Journal of Clinical Microbiology*, **38**, 4633-4636.

Gebreyes, W.A., Davies, P.R., Turkson, P-K., Morrow, W.E.M., Funk, J.A. and Altier, C. (2004a): *Salmonella enterica* serovars from pigs on farms and after slaughter and validity of using bacteriologic data to define herd *Salmonella* status. *Journal of Food Protection*, **67**, 691-697.

Gebreyes, W.A., Davies, P.R., Turkson, P-K., Morrow, W.E.M., Funk, J.A., Altier, C., Thakur, S. (2004b): Characterization of antimicrobial-resistant phenotypes and genotypes among *Salmonella enterica* recovered from pigs on farms, from transport trucks, and from pigs after slaughter. *Journal of Food Protection*, **67** (4), 698-705.

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

Gupta, B. R. and Verma, J. C. (1990): Prevalence of *Salmonella* in farm animals and birds in India. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, **11**(3 and 4), 169-74

Hald, T., Wegener, H.C. (1999): Quantitative assessment of the sources of human salmonellosis attributable to pork. In: Proceedings of the Third International Symposium on the Epidemiology and Control of Salmonella in Pork. Washington, DC, USA, pp. 200-205.

Hald, T., Wingstrand, A., Swanenburg, M., Von Altrock, A., and Thorberg, B.-M. (2003): The occurrence and epidemiology of *Salmonella* in European pig slaughterhouses. *Epidemiology and Infection*, **131**, 1187-1203.

Hendriksen SWM, Orsel K, Wagenaar JA, Miko A, and van Duijkeren E. (2004): Animal-to-human transmission of Salmonella Typhimurium DT104A variant. *Emerging Infectious Diseases*, **10** (12), 2225-2227.

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

Hsueh P.R, Teng L.J, Tseng S.P, Chang C.F, Wan J.H, Yan J.J, *et al.* (2004): Ciprofloxacin-resistant *Salmonella enterica* Typhimurium and Choleraesuis from Pigs to Humans, Taiwan. *Emerging Infectious Diseases*.**10**(1) [access date May 20, 05]. Available from: URL: http://www.cdc.gov/ncidod/EID/vol10no1/03-0371.htm

Hurd, H. S., McKean, J. D., Griffith, R. W., Wesley, I. V., and Rostagno, M.H. (2002): *Salmonella enterica* infections in market swine with and without transport and holding. *Applied and Environmental Microbiology*, **68**, 2376-2381.

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

Isaacson, R.E., Firkins, L.D., Weigel, R.M., Zuckermann, F.A., and DiPietro, J.A. (1999): Effect of transportation and feed withdrawal on shedding of *S.* Typhimurium among experimentally infected pigs. *American Journal of Veterinary Research*, **60**(9), 1155-1158.

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

Le Minor, L., and Popoff, M. Y. (1987): Request for an opinion, Designation of *Salmonella enterica* sp. nov., nom. rev., as the type and only species of the genus *Salmonella*. *International Journal of Systematic Bacteriology*, **37**, 465-468.

Letellier, A., Messier, S., Paré, J., Ménard, J., and Quessy, S. (1999): Distribution of *Salmonella* in swine herds in Québec. *Veterinary Microbiology*, **67**, 299-306.

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

Mache, A., Mengistu, Y. and Cowley, S. (1997): *Salmonella* serogroups identified from adult diarrhoeal out-patients 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.

Maslow, J. N., Mulligan M. E. and Arbeit, D. R. (1993). Molecular epidemiology: Application of contemporary techniques to the typing of microorganisms. *Clinical Infectious Diseases*, **17**: 153-164.

Merck, E. (1992): Microbiology Manual. Darmstadt, Germany.

Mølbak K., Baggesen D., Aarestrup F., Ebbesen J., Engberg J., Frydendahl K., Gerner-Smidt P., Petersen A. and Wegener H. (1999): An outbreak of multidrug-resistant, quinolone-resistant Salmonella enterica serotype typhimurium DT104. *The New England Journal of Medicine*, **341**,1420-1425.

Molla, B., Alemayehu, D. and Salah, W. (2003a): Sources and distribution of *Salmonella* serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997-2002, *The Ethiopian Journal of Health Development*, **17**, 63-70.

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

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

Molla, B., Mohammed, A., and Salah, W. (2004a): *Salmonella* prevalence and distribution of serotypes in apparently healthy slaughtered camels (*Camelus dromedarius*) in eastern Ethiopia. *Tropical Animal Health and Production*, **36**, 451-458.

Molla, B., Salah, W., Alemayehu, D., and Mohammed, A. (2004b): Antimicrobial resistance pattern of *Salmonella* serotypes isolated from apparently healthy slaughtered camels (*Camelus dromedarius*) in eastern Ethiopia. *Berliner und Munchener 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.

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.

National Committee for Clinical Laboratory Standards (1999): 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.

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 Africa*, **48**, 19-24.

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

Patterson, S., and Isaacson, R. E. (2003): Genetics and pathogenesis of *Salmonella*. 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 Production*, **13**, 203-207.

Popoff, M. Y., and Le Minor, L. (1997): Taxonomy of the genus *Salmonella*: Changes in serovars nomenclature. In: Antigenic formulas of the *Salmonella* serovars, 7th revision. WHO collaborating center for reference and research on *Salmonella*. Pasteur Institute, Paris, France.

Quinn, P. J., Carter, M. E., Markey, B., and Carter, G.R. (1999): *Enterobacteriaceae*. In: Clinical Veterinary Microbiology. Mosby 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.

Rajic, A., Keenliside, J., McFall, M.E., Deckert, A.E., Muckle, A.C., O'Connor, B.P., Manninen, K., Dewey, C.E., and McEwen, S.A. (2005): Longitudinal study of *Salmonella* species in 90 Alberta swine finishing farms, *Veterinary Microbiology*, **105**, 47-56.

Roof, M. B.; Roth, J. and Kramer, T. T. (1993). Porcine Salmonellosis: Characterization, Immunity, and Potential Vaccines. The Compendium Collection: Infectious Disease in Food Animal Practice. Veterinary Learning Systems. Trenton, New Jersey. pp 191-201.

Rowe, T.A., Leonard, F.C., Kelly, G., Lynch, P.B., Egan, J., Quirke, A.M., and Quinn, P.J. (2003): *Salmonella* serotypes present on a sample of Irish pig farms. *Veterinary Record*, **153**, 453-456.

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

Schwartz, K. J. (1993). Salmonellosis in Swine. The Compendium Collection: Infectious Disease in Food Animal Practice. Veterinary Learning Systems. Trenton, New Jersey. pp 202-209.

Seifert, H. S. H. (1996): Tropical Animal Heath. Dordrecht: Kluwer Academic Publishers. pp 368-371.

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

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

SPSS (2002): Statistical Packages for Social Sciences, SPSS for windows (Release 11.5.0), SPSS Inc., 1989-2002.

Tauxe R. V. (1997): Emerging food-borne diseases: an evolving public health challenge. *Emerging Infectious Diseases*, **3**, 425–34.

Tegegne, M., and Ashenafi, M. (1998): Microbial load and incidence of *Salmonella* spp. in 'kitfo', a traditional Ethiopian spiced, minced meat dish. *Ethiopian Journal of Health Development*, **12**, 130-140.

Thorberg, B.-M., and Engvall, A. (2001): Incidence of *Salmonella* in five Swedish slaughterhouses. *Journal of Food Protection*, **64** (4), 542-545.

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 Munchener 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 Africa*, **50**, 86-95.

Uyttendaele, M. R., Debevere, J. M., Lips, R. M., Neysts, K. D. (1998): Prevalence of Salmonella in poultry carcasses and their products in Belgium. *International Journal of Food Microbiology*, **40**, 1-8.

Uzzau, S., Brown, D. J., Wallis, T., Rubino, S., Leori, S., Bernard, S., Casadesus, J., Platt, D. J., and Olsen, J. E. (2000): Host adapted serotypes of *Salmonella enterica*: review. *Epidemiology and Infection*, **125**, 229-255.

van der Wolf, P. J., Bongers, J. H., Elbers, A. R. W., Franssen, F. M. M. C., Hunneman, A. C. Z, van Exsel, A. C. A., and Tielen, M. J. M. (1999): *Salmonella* infections in finishing pigs in The Netherlands: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection. *Veterinary Microbiology*, **67**, 263-275.

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.

Ward L. R., De Sa. J. D. H., and Rowe. B. (1987): A phage-typing scheme for *Salmonella* Enteritidis. *Epidemiology and Infection*, **99**, 291-294.

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., 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.

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, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D. G., Wagner, D., and Meng, J. (2001): Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D. C. area. *Applied and Environmental Microbiology*, **67**, 5431-5436.

8. APPENDICES

Appendix I: 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 casein10.0; 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^{TM}, 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 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 Petri dishes.

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 121oC for 15 minutes. Cool in a slanted position so that deep butts are formed.

7. Lysine iron agar (LIA) (Difco™, 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°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°C for 15 minutes. Cool to 50°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.

Appendix II: Working sheet for recording colony morphology and biochemical reactions of presumptive *Salmonella* isolates

Sample No.	Date of collection	Sample type	Farm/origin	sex	media		Colony characteristics	isolate no.	TSI				LIA			Urase	Citrate	Rambach
					enrichment	plating			lactose &/or Sucrose	Dextrose	H2S	gas	H2S	lysine decarboxylase	lysine deaminase			

9. Rambach®-agar (Merck, Darmstadt, Germany)

Composition (g/liter): Peptone 8.0; sodium chloride 5.0; sodium desoxycholate 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.

10. 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 liter 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.

11. 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 litre distilled water and heat ot boiling to dissolve completely. Sterilize in the autoclave for 15 minutes at 121°C.

Appendix II: Working sheet for recording colony morphology and biochemical reactions of presumptive *Salmonella* isolates

Sample No.	Date of collection	Sample type	Farm/origin	sex	media		Colony characteristics	isolate no.	TSI				LIA			Urase	Citrate	Rambach
					enrichment	plating			lactose &/or Sucrose	Dextrose	H2S	gas	H2S	lysine decarboxylase	lysine deaminase			

9. CURRICULUM VITAE

A. Biographical Data:

Name	Kassaye Aragaw
Date of birth	February 15, 1970
Place of birth	Negele Borena, Ethiopia
Marital status	Married
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Researcher at Sheno Agricultural Research Center of the Amhara Regional Agricultural Research Institute

B. Educational background

Year	School Attended
1977- 1984	Addola Elementary and juniour school, Kibre Mengist
1984 -1988	Kibre Mengist senior secondary school, Kibre Mengist
1988 -1994	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia

C. Work Experience

May 1996 to July 2000	Government employed field veterinarian in two woredas of South Wollo zone of the Amhara National Regional State
Since July 2000 to today	Assistant researcher at Sheno Agricultural Research Center of the Amhara Regional Agricultural Research Institute

D. Research output/Technical paper

1.Factors affecting hematological profiles in three Ethiopian indigenous goat breeds. M. Tibbo, Y. Jibril, M. Woldemeskel, F. Dawo, **K. Aragaw**, and J.E.O. Rege. *Intern J Appl Res Vet Med • Vol. 2, No. 4, 2004*

2. Effects of anthelmintics and supplementaion on productivity of Menz and Menz-Awassi crossbred sheep with sub-clinical helminthosis. Markos Tibbo, **Kassaye Aragaw** and Assefa Deressa. *Ethiop. Vet. J. Vol. 8, No. 2, 2004*

2. Study on host resistance to natural tick infestation in Friesian and indigenous zebu cattle. (DVM thesis presented to FVM, AAU, 1995)

E. Membership to professional societies

Member of Ethiopian Veterinary Association and Ethiopian Society of Animal Sciences.

F. Language

Amharic	Mother Tongue
English	Writing and speaking

G. Computer Skill

MS Dose, MS Word, MS Excel, MS Access

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 my approval as University advisor.

Dr.Bayleyegn Molla (Associate Professor)

_____

Salmonella In Apparently Healthy Slaughtered Swine In Assis Ababa, Ethiopia

Kassaye Aragaw