ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

A CROSS-SECTIONAL STUDY ON *SALMONELLA* IN APPARENTLY HEALTHY SLAUGHTERED SHEEP AND GOATS AT ADDIS ABABA AND MODJO ABATTOIRS, ETHIOPIA

By
WASSIE MOLLA ABEBE

JUNE, 2004
DEBRE ZEIT, ETHIOPIA

ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

A CROSS-SECTIONAL STUDY ON *SALMONELLA* IN APPARENTLY HEALTHY SLAUGHTERED SHEEP AND GOATS AT ADDIS ABABA AND MODJO ABATTOIRS, 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
WASSIE MOLLA ABEBE

JUNE, 2004
DEBRE ZEIT, ETHIOPIA

A CROSS-SECTIONAL STUDY ON *SALMONELLA* IN APPARENTLY HEALTHY SLAUGHTERED SHEEP AND GOATS AT ADDIS ABABA AND MODJO ABATTOIRS, ETHIOPIA

By

WASSIE MOLLA ABEBE

Board of Examiners	Signature
Prof. Ph. Dorchies	________________________
Prof. Feseha Gebreab	________________________
Dr. Wondwossen Abebe Gebreyes	________________________
Dr. Giles Innocent	________________________
Dr. Andy Cately	________________________
Dr. David Barrett	________________________

Academic Advisor

Dr. Bayleyegn Molla ________________________

DECLARATION

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)

____

ACKNOWLEDGEMENTS

I am greatly indebted to my advisor Dr. Bayleyegn Molla. Without his technical and professional advice, encouragement, material provision and time devotion to correct the paper, this thesis would not have been completed.

I would like to extend my gratitude to Dr. Daniel Alemayehu for his excellent technical assistance.

I am grateful to Ato Abebe Tessema, manager of Modjo modern export abattoir, for allowing me to carry out my study in the abattoir and for his assistance and cooperation during the time of my work in the abattoir. I also thank the Modjo modern export abattoir personnel for their cooperation during sampling.

I would like to thank the Addis Ababa Abattoir Enterprise for the permission rendered to carry out the work in the abattoir. I also extend my gratitude to Dr. Bitew Getahun and the abattoir personnel for their generous cooperation.

Special thanks goes to Dr. Anne Muckle, head of the Laboratory for Foodborne Zoonoses, Health Canada, Dr. Cornelis Poppe, Ms Linda Cole and Ms Betty Welkie at the OIÉ Reference Laboratory for Salmonellosis, Health Canada, Canada for the complete serotyping, phage typing and antibiogram test of the *Salmonella* isolates.

It is my pleasure to thank Dr. Mattheiu Lesnoff for his kind assistance in statistical analysis of my data.

I wish to thank Drs. Getahun Ejeta, Ermias Woldemariam, Alehegn Wubete, Wudu Temesgen, and Araya Mengistu for their technical assistance in this study.The generous cooperation of the technical stuff of the Microbiology Laboratory of FVM is gratefully acknowledged.

Last but not least I sincerely acknowledged my wife W/o Bethelihem Mesfin for her tremendous support throughout my study.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS .... **I**
LIST OF TABLES .... **IV**
LIST OF FIGURES .... **V**
LIST OF APPENDICES .... **VI**
LIST OF ABBREVIATIONS .... **VII**
ABSTRACT .... **IX**
1. INTRODUCTION **1**
2. LITERATURE REVIEW **5**
2.1. The Genus *Salmonella* **5**
2.1.1. Taxonomy and classification 5
2.1.2. Morphology 6
2.1.3. Antigenic structure and notation 7
2.1.4. Physiology and effects of environmental factors 9
2.1.5. Biochemical characteristics 10
2.1.6. Typing of *Salmonella* 10
2.1.7. Antimicrobial resistance 12
2.2. Epidemiology **13**
2.2.1. Prevalence of *Salmonella* in sheep and goats 14
2.2.2. Sources of infection and mode of transmission 16
2.2.3. Predisposing factors to clinical salmonellosis 17
2.3. Pathogenesis **18**
2.4. Salmonellosis in Sheep and Goats **19**
2. 5. Diagnostic Methods for the Detection of *Salmonella* **20**
2.5.1. Bacteriological examinations 20
2.5.2. Biochemical tests 22
2.5.3. Serological tests 22
2.6. Public Health Importance of Salmonellosis **23**
2.7. Economic Importance of Salmonellosis **25**
2.8. Status of Salmonellosis in Ethiopia **25**
2.9. Prevention and Control **27**
3. MATERIALS AND METHODS **29**
3. 1. Study Areas **29**
3. 2. Study Population **29**
3.3. Study Design **30**
3.4. Sampling **31**
3.4.1. Sample size 31
3.4.2. Sampling procedures 31
3.5. Isolation and Identification of *Salmonella* **32**
3.5.1. Pre-enrichment 32
3.5.2. Selective enrichment 34
3.5.3. Selective platting and identification 34
3.5.4. Confirmation 34

3.5.5. *Salmonella* serotyping 36
3.5.6. Phage typing 37
3.5.7. Resistance to antimicrobial agents 37
3.6. Data Management and Analysis 40

4. RESULTS 41

4.1. Prevalence of *Salmonella* 41
4.2. Isolation of *Salmonella* from Different Sources 42
4.3. *Salmonella* Serotypes Isolated 45
4.4. *Salmonella* Phage Types Identified 47
4.5. Antimicrobial Resistance of *Salmonella* Isolates 48

5. DISCUSSION 52

5.1. Prevalence 52
5.2. Distribution of *Salmonella* Serotypes 58
5.3. Antimicrobial Resistance Pattern of *Salmonella* Isolates 60
5.4. Synopsis 61

6. CONCLUSIONS AND RECOMMENDATIONS 63

7. REFERENCES 65

8. APPENDICES 77

9. CURRICULUM VITAE 85

LIST OF TABLES

Page

Table 1. Antigens of some selected *Salmonella* serotypes 8

Table 2. Prevalence of *Salmonella* in sheep and goats in different countries 15

Table 3. Antimicrobials and concentrations used to test susceptibility of *Salmonella* isolates..... 38

Table 4. Occurrence of *Salmonella* in apparently healthy slaughtered sheep and goats at Addis Ababa and Modjo abattoirs 41

Table 5. Association of species and abattoirs with *Salmonella* isolation in sheep and goats....... 42

Table 6. Isolation of *Salmonella* in tissue and faecal samples of apparently healthy slaughtered sheep at Addis Ababa and Modjo abattoirs 43

Table 7. *Salmonella* isolation in tissue and faecal samples of apparently healthy slaughtered goats at Addis Ababa and Modjo abattoirs........ 45

Table 8. Distribution of *Salmonella* serotypes by animal species and abattoir source 46

Table 9. *Salmonella* serotypes isolated from sheep and goats........ 47

Table 10. Distribution of *Salmonella* serotypes and phage types in various sample types of sheep and goats 48

Table 11. Distribution of antimicrobial resistance of *Salmonella* isolates by source 49

Table 12. Resistance of *Salmonella* isolates from sheep and goats by antimicrobial type 50

Table 13. Mutlidrug resistance pattern of *Salmonella* isolates........ 51

Table 14. Resistance of *Salmonella* isolates by number of antimicrobials 51

LIST OF FIGURES

Page

Fig. 1. *Salmonella* cycling and recycling 17
Fig.2. Mode of transmission of *Salmonella* (except *Salmonella* Typhi and the Paratyphoid serotypes).. 24
Fig. 3. Flow diagram showing the horizontal method for the detection of *Salmonella* (ISO 6579, 1998).. 33
Fig. 4. *Salmonella* isolation in different sample types 44

LIST OF APPENDICES

Page

Appendix I. Media used and preparations for the isolation and identification of *Salmonella* 77

Appendix II. *Salmonella* isolation in tissue and fecal samples of sheep from Addis Ababa and Modjo abattoirs 81

Appendix III. *Salmonella* isolation in tissue and fecal samples of goats from Addis Ababa and Modjo abattoirs 83

LIST OF ABBREVIATIONS

AAU	Addis Ababa University
Abm	Abdominal muscle
a_w	Water activity
BPLS	Brilliant green- phenol red- lactose sucrose agar
BPW	Buffered peptone water
CACC	Central Agricultural Census Commission
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
Dev	Deviance
Df	Degrees of freedom
Dim	Diaphragmatic muscle
DVM	Doctor of Veterinary Medicine
FVM	Faculty of Veterinary Medicine
gm	Gram
H	Flagella antigen
h	Hour
HACCP	Hazard Analysis Critical Control Point
H_2S	Hydrogen sulphide
ICMSF	International Commission on Microbiological Specifications
ILRI	International Livestock Research Institute
ISO	International Organization for Standardization
K(Vi)	Capsular antigen
MLn	Mesenteric lymph nodes
µg	Microgram
ml	Millilitre
mm	Millimetre
MR-VP	Methyl red-Voges-Proskauer
MS	Microsoft
MSc	Master of science
NCCLS	National Committee for Clinical Laboratory Standards
NMCA	National Meteorological Centre Agency
O	Somatic antigen
^{O}C	Degree Celsius

OIÉ	Office International des Épizooties
OR	Odds ratio
PT	Phage type
Resid.	Residual
RV	Rappaport-Vassilliadis
SC	Selenite cystine
TSI	Triple sugar iron agar
Var.	Variety
WHO	World Health Organization
XLD	Xylose lysine desoxycholate agar

ABSTRACT

The present study was undertaken on apparently healthy slaughtered sheep and goats, and apparently healthy abattoir personnel at Addis Ababa public and Modjo modern export abattoirs from September, 2003 to February, 2004. The objectives of the study were to determine the prevalence and distribution of *Salmonella* in sheep and goats and abattoir personnel, to identify and characterize the prevalent *Salmonella* serotypes and to determine the antimicrobial resistance of *Salmonella* isolates to selected antimicrobial agents. A total of 1224 samples consisting of faeces (n=204), mesenteric lymph nodes (n=204), liver (n=204), spleen (n=204), abdominal (n=204) and diaphragmatic muscles (n=204) samples from 104 sheep and 100 goats and 117 stool samples from abattoir personnel were collected. The samples were examined for the presence of *Salmonella* following the techniques recommended by the International Organization for Standardization (ISO 6579, 1998).

Salmonellae were isolated from 12 of 104 (11.5% (95% CI = 6.1-19.3)) sheep and 3 of 100 (3% (95% CI = 0.6-8.5)) goats and none of the samples from abattoir personnel were positive for *Salmonella*. The frequency of isolation and distribution of *Salmonella* in different tissue and faecal samples of sheep and goats was analyzed. Of the total 624 samples examined from apparently healthy slaughtered sheep, 18 (2.9%) were *Salmonella* positive. Salmonellae were isolated from 4.8% faecal, 7.7% mesenteric lymph nodes, 1.9% liver and 1.9% abdominal muscle samples. Only one isolate was found from spleen and *Salmonella* was not detected from diaphragmatic muscle samples. Four (0.7%) *Salmonella* positive samples were detected from a total of 600 samples from apparently healthy slaughtered goats. *Salmonella* was isolated from 2% faecal and 2% mesenteric lymph nodes samples whereas in other organs *Salmonella* was not detected. In both species, the number of positive specimens was higher for faeces and mesenteric lymph nodes samples than others.

Serotypes and phage types of *Salmonella* strains isolated from sheep and goats were identified. All the 22 *Salmonella* isolates were serotyped and serotypes of *S*. Typhimurium, *S*. Heidelberg, *S*. Typhimurium var. Copenhagen and *S*. Enteritidis strains were further phage typed. A total of 9 different serotypes were identified from sheep (n=7) and goats (n=3), of which *S*. Typhimurium (36.4%) and *S*. Heidelberg (18.2%) were the most prevalent serotype followed by *S*. Give, *S*. Poona and *S*. Reading (each 9.1%). Other serotypes including *S*. Typhimurium var. Copenhagen, *S*. Enteritidis, *S*. Niederoderwitz and *S*. I: 6, 7, 14:-: enz15 were also detected from sheep and goats. The most common serotypes recovered in sheep

were *S.* Typhimurium (38.9%) and *S.* Heidelberg (22.2%) and in goats it was *S.* Poona (50.0%). Three serotypes, *S.* Poona, *S.* I: 6, 7, 14:-:enz15 and *S.* Niededrowtiz were reported for the first time in Ethiopia.

The phage types identified include *S.* Typhimurium phage type 46, *S.* Typhimurium phage type 193, *S.* Typhimurium phage type 2, *S.* Typhimurium phage type 79, *S.* Typhimurium phage type U285, *S.* Typhimurium var. Copenhagen phage type 104, *S.* Enteritidis phage type 5a and *S.* Heidelberg phage type atypical. Of which *S.* Heidelberg phage type atypical was the most common phage type in sheep. *Salmonella* Typhimurium phage type 46 was the most prevalent phage type of *S.* Typimurium phage types in sheep whereas *S.* Typhimurium phage type 193 was found both in sheep and goats.

All isolates were tested for susceptibility to a group of 24 selected antimicrobials. Out of the 22 isolates tested, 7 (31.8%) were multiple resistant from two to up to nine antimicrobials. Among the isolates, only *S.* Typhimurium (62.5%) and *S.* Reading (100%) displayed multiple resistances to ten different antimicrobials. *Salmonella* Typhimurium isolates were resistant to ampicillin and cephalothin (each 50.0%), sulfisoxazole, streptomycin, sulfamethoxazol-trimethoprim and trimethoprim (each 25.0%), amoxicillin/clavulanic acid, chloramphenicol, spectinomycin and tetracycline (each 12.5%). However, *S.* Reading isolates showed resistance only to streptomycin, sulfisoxazole and tetracycline (each 100%).

The results of this study showed the potential risk of sheep and goats as sources of pathogenic and multi-drug resistant *Salmonella* serotypes for humans in the study area. This is a significant threat to public health particularly to those who have direct or indirect contact to sheep and goats and consumers who have the habit of eating sheep and goats meat or meat products either raw or under cooked. These findings stressed the need for implementation of *Salmonella* control and prevention strategies from farm production to consumption of animal and animal products.

Key words: Sheep, goats, abattoir personnel, *Salmonella*, serotypes, prevalence, antimicrobial resistance, Addis Ababa, Modjo

1. INTRODUCTION

Ethiopia has about 14.7 million sheep and 13.7 million goats (CACC, 2003). Small ruminants are important livestock resources in Ethiopia, providing 35% (148,850 tons) of meat consumption (2.7 kg per capita per year) and 14% of milk consumption (Asfaw, 1997). The contribution of livestock to the productivity and sustainability of smallholder farming system is severely compromised by ill health and diseases. Diseases reduce incomes directly by causing considerable livestock losses and indirectly by necessitating health restriction on exports. Infectious disease including *Salmonella* still poses major threats to livestock production, human health and export of animals and animal products in developing countries.

Salmonellosis is an infectious and economically important disease of humans and animals caused by *Salmonella* organisms (Radostits *et al.*, 1994; OIÉ, 2000). *Salmonella* infection in animals and humans occurs mostly through the ingestion of contaminated feed/food and water or through contact with the contaminated excretions of latently infected animals; shedders always playing a major role in the dissemination of the organisms (Seifert, 1996).

Although primarily intestinal bacteria, *Salmonella* are widespread 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 (Wray and Davies, 2003).

Infection of animals with various serotypes of *Salmonella* sometimes results in serious disease and constitutes a vast reservoir for the disease in humans. The interplay of *Salmonella* with its host takes a variety of forms, including remarkable host specificity, inapparent infections, recovered carriers, enteritis, septicaemia, abortion, and combinations of disease syndromes. Salmonellae are readily transferred from animal to animal, animal to humans, and human to human by direct or indirect pathways (Clarke and Gyles, 1993).

Salmonellosis is globally one of the major foodborne infections in humans primarily caused by *Salmonella* contaminated meats. The disease situation in humans has become increasingly worse during the last decade (Radostits *et al.*, 1994; Wierup, 1995). According to WHO the situation has reached alarming proportions in several countries (Wierup, 1995). It is clear that the occurrence of salmonellae in the global food chain and its current and project repercussion

on human health are cause for concern. Unless significant changes in agriculture and agricultural practices are implemented, human foodborne salmonellosis will prevail in the next century (D'Aoust, 1997).

Interest in *Salmonella* pathogen has heightened in recent years due to the increased susceptibility of AIDS patients to salmonellosis (Clarke and Gyles, 1993). Salmonellosis is not only responsible for human illness, but also creates greater problems in international trade of food and tourism. *Salmonella* has been distributed worldwide through international trade of animals, animal products and feeds (D'Aoust, 1994; Seifert, 1996). The costs involved in human salmonellosis linked to foods of animal origin are substantial (medical expenses, loss in economic productivity and income) (Person and Jendteg, 1992; Bryan and Doyle, 1995; Stohr, 1995). Salmonellosis is also a significant cause of economic loss in farm animals because of the costs of clinical disease which include deaths, diagnosis and treatment of clinical cases, diagnostic laboratory costs, the costs of cleaning and disinfection and the costs of control and prevention (Radostits *et al*., 1994).

In modern livestock production, the world over relies on the use of antimicrobial substance, not only for the treatment of infection but also for the promotion of growth and the prevention of disease (Wray *et al*., 1993). Current global trends depicting sustained increases in the number of antibiotic-resistant *Salmonella* strains in humans and farm animals are most disquieting (D'Aoust, 1997). The widespread administration of prophylactic doses of medically important antibiotics to reared animals species may promote on-farm selection of antibiotic-resistant strains and markedly increase the human health risks associated with handling and consumption of contaminated meat products. In many countries of Africa including Ethiopia, high incidence of antibiotic resistance has been observed in *Salmonella* (Molla *et a*l., 1999b; Mache, 2002).

It has been reported that sheep and goats harbor a wide range of *Salmonella* serotypes and so act as reservoirs of paramount epidemiological importance in human salmonellosis. Workers from many countries have demonstrated the presence of salmonellae in the mesenteric lymph nodes, liver, spleen, faeces and carcasses of slaughtered sheep and goats (Kumar *et al*., 1973 in India; Doutre and Boche, 1976 in Senegal; Kane, 1979 in New Zealand; Nabbut and Al-Nakhli, 1982 in Saudi Arabia; Abdel-Ghani *et al*., 1987 in Egypt; Kotova *et al*., 1988 in Rassia; Das *et al*., 1990 in India; Smith and Sherman, 1994).

Contamination of red meat by salmonellae may occur at the abattoirs from symptomless animal execrators, contaminated abattoir equipment and floors. The *Salmonella* organisms can gain access to meat at any stage during butchering. It is obvious that presence of salmonellae in faeces and the mesenteric lymph nodes, which are frequently incised during the inspection process, constitutes a source for contamination of the red meat and other edible parts of the carcasses (Smeltzer *et al.,* 1980; Moo *et al*., 1980; Nabbut and Al-Nakhli, 1982).

Diagnosis of salmonellosis is based on the isolation of the organism either from tissues collected aseptically at the necropsy or from faeces, rectal swabs or environmental samples. *Salmonella* can be isolated using standard cultural techniques and various biochemical and serological tests. The biochemical and serological tests can be applied to the pure culture to produce definitive confirmation of an isolated strain (OIÉ, 2000). It will not be possible to eradicate salmonellosis from any country. However, it should be possible to control the incidence of disease in domestic and farm animals and also in the human population simply by ensuring that food and food supplements do not contain salmonellae (Doyle and Cliver, 1990; Baired-Parker, 1990).

Previous studies conducted in Ethiopia on salmonellosis indicated the existence of the infection in various animal species (poultry, cattle, camels, sheep and goats), retail food items (minced beef, chicken meat and offal) as well as in humans (Mache *et al*., 1997; Molla *et al*., 1999a; Nyeleti *et al.,* 2000; Molla *et al*., 2003a; Woldemariam, 2003). However, the information on sheep and goat salmonellosis prevalence, distribution, serotypes involved and the zoonotic importance of sheep and goat salmonellosis is limited. Woldemariam (2003) reported a prevalence of 6.4% in sheep and 16.7% in goats at Elfora Debre Zeit abattoir and Ejeta *et al*. (2004) reported a 14.1% *Salmonella* contamination rate of mutton at Addis Ababa supermarkets.

This study was therefore undertaken on apparently healthy slaughtered sheep and goats and apparently healthy abattoir personnel at Addis Ababa public abattoir and Modjo modern export abattoir. Liver, spleen, mesenteric lymph nodes, abdominal and diaphragm muscles and faecal samples from apparently healthy slaughtered sheep and goats, and stool samples from apparently healthy abattoir personnel were collected and examined for the presence of *Salmonella* with the following objectives:

- To determine the prevalence and distribution of *Salmonella* in sheep and goats,

- To identify and characterize the prevalent *Salmonella* serotypes,
- To determine the infection level in abattoir personnel and
- To determine the antimicrobial resistance of *Salmonella* isolated from apparently healthy slaughtered sheep and goats and abattoir personnel.

2. LITERATURE REVIEW

Salmonellosis is an infectious disease of humans and all animal species world wide (Radostits *et al*., 1994; OIÉ, 2000). Infection of animals with various serotypes of salmonellae can result in serious clinical disease particularly in young, elder and stressed animals and always constitutes the vast reservoir for human infections. Clinically, salmonellosis can be manifested by one of three major syndromes: peracute septicemia, acute or chronic enteritis (Radostits *et al*., 1994; Aiello and Mays, 1998).

2.1. The Genus *Salmonella*

2.1.1. Taxonomy and classification

The genus *Salmonella* is facultative anaerobic Gram-negative rods belonging to the family *Enterobacteriacae* and includes a large number of serotypes of human beings as well as mammals, which are antigenically related to one another. Currently more than 2435 serotypes of *Salmonella* are known, with the exception of a handful of these (such as *S.* Typhi, *S.* Paratyphi) all are primary pathogens of animals with capability to infect human beings also (Bhatia and Ichhpujani, 1994; D'Aoust, 1997; Popoff and Le Minor, 1997).

The genus *Salmonella* consists of only two species: *S. enterica* and *S. bongori* (formerly called *S. enterica* subspecies *bongori*). *Salmonella enterica* is divided into six subspecies (Popoff and Le Minor, 1997; OIÉ, 2000). Subspecies are distinguished by certain biochemical characteristics and some of which correspond to the previous subgenera. These subspecies are: Subspecies I (*S. enterica* subspecies *enterica)*, subspecies II (*S. enterica* subspecies *salamae*), subspecies IIIa (*S. enterica* subspecies *arizonae*), subspecies IIIb (*S. enterica* subspecies *diarizonae*), subspecies IV (*S. enterica* subspecies *houtenae)*, and subspeciesVI (*S. enterica* subspecies *indica*). Of the serovars of this genus that had been identified up to now, only 20 belonged to *S. bongori* and the remaining to *S. enterica* (Bhatia and Ichhpujani, 1994; D'Aoust, 1997; Popoff and Le Minor, 1997). Subspecies I includes the causative agents of typhoid and paratyphoid as well as it contains most of the salmonellae that are significant animal pathogens (Bhatia and Ichhpujani, 1994; Quinn *et al*., 1994).

The naming of *Salmonella* is done by international agreement. Under this system, a serovar is usually named after the place where it was first isolated. In clinical practice the subspecies name does not need to be indicated as only serovars of subspecies *enterica* bear a name: Typhimurium, London or Montevideo are serovars of subspecies *enterica.* The name *Salmonella* serovar Typhimurium or *Salmonella* Typhimurium may be used for routine practice. The first letter of the serovar name should be written in capital letter and must no longer be italicized. Serovars of other subspecies of *S. enterica* and those of *S. bongori* are designated only by their antigenic formula (Popoff and Le Minor, 1997; Jay, 2000).

Based on their association with human and animal hosts *Salmonella* can be classified into three main groups (Radostits *et al.,* 1994). The first group comprises *Salmonella* Typhi and Paratyphi A and C, which infect only man and are spread either directly or indirectly (via food and water) from person to person (WHO, 1988; Radostits *et al.*, 1994). The second group includes serovars that are host adapted for particular species of vertebrates, example *S.* Gallinarum in poultry, *S.* Dublin in cattle, *S.* Abortus equi in horse, *S.* Abortus ovis in sheep and *S.* Choleraesuis and *S.* Typhisuis in swine. Some of these are also pathogenic for man (especially *S.* Dublin and *S.* Choleraesuis). The third group contains the majority of other *Salmonella* serovars with no particular host preference that infect both animals and man. Among this third reservoir of serovars are principal agents of salmonellosis that occurs today (Jay, 2000).

2.1.2. Morphology

The morphology of salmonellae is similar to that of other enterobacteria. They are Gram-negative bacilli which are non-acid fast and non-sporing. These organisms usually occur as short rods, measuring 2-4 micrometer in length and 0.5 micrometer in diameter. Occasionally, they develop into longer pleomorphic forms or very short coccobacilli after prolonged culture on laboratory media. With the exception of *S.* Pullorum and *S.* Gallinarum, all strains normally possess peritrichous flagella and are actively motile. Occasionally non motile variants may be encountered in any serotype as a result of the occurrence of dysfunctional flagella. Many serotypes are also known to develop fimbriae (Old, 1990; Bhatia and Ichhpujani, 1994; Quinn *et al.*, 1994; D’Aoust, 1997).

2.1.3. Antigenic structure and notation

Like other *Enterobacteriaceae* salmonellae possess somatic, flagellar and capsular antigens. Somatic antigens are designated (O) antigens, flagellar antigens (H) antigens and capsular antigens (K) antigens. The somatic antigen is composed of a lipopolysaccharide protein complex (LPS) on the external surface of the bacteria outer membrane. The heat stable O antigens are classified as major or minor antigens. The polysaccharides determine the serological specificity. The flagellar antigen is part of the flagella of the bacterium and proteinacious. The specificity is determined by the pattern of amino acids and it is thermo-labile. It is present in one or both of two phases. The capsular antigen lies at the periphery of the cell wall and prevents access of anti-O agglutinins (antibodies) to their homologous somatic antigen. The K antigen differs from ordinary O antigens in being destroyed by heating for one hour at 60^0C and by dilute acid and phenole. Agglutination tests with absorbed antisera for different O and H antigens form the bases for serological classification of the salmonellae (Old, 1990; Jawetz *et al*., 1995; Seifert, 1996; D'Aoust, 1997). Table 1 shows the antigens of some selected *Salmonella* serotypes of veterinary and public health importance.

The identification and labelling of the *salmonella* antigens was initiated by White and continued and extended by Kauffmann whose terminology was adopted for general use (Old, 1990). The antigenic formula consists of three parts, describing the somatic (O) antigen, the phase-1 H antigen and the phase-2 H antigen, in this order. The three parts are separated by colons and the components of each part by commas. The somatic antigens (O) are given Arabic numerals. The phase-1 H antigens are designated a to z, a series that is complete except for j; those described after z were designated as z with subscripts, e.g., z_1 to z_{68}. The phase-2 H antigens are designated by Arabic numerals (1-12) but phase 2 may also contain antigenic components of the e series or of the z series. Some H antigens appear in phase 1 only, others in phase 2 only; a few types-e, l, w and some z factors appear in both phases (Old, 1990).

Certain somatic factors are present in some members only of particular serotypes. This is indicated by placing the factor within a square bracket in the antigenic formula. Some somatic factors underlined. This is because they are determined by the presence of a phage (Old, 1990; Quinn *et al*., 1994).

Table 1. Antigens of some selected *Salmonella* serotypes

Serotypes	Serogroup	Somatic (O) antigens	Flagella (H) antigens	
			Phase 1	Phase 2
S. Paratyphi A	A	$\underline{1}$, 2, 12	a	[1, 5]
S. Typhimurium	B	$\underline{1}$, 4, [5], 12	i	1, 2
S. Derby	B	$\underline{1}$, 4, [5], 12	f, g	[1, 2]
S. Agona	B	4, 12	f, g, s	-
S. Saintpaul	B	$\underline{1}$, 4, [5], 12	e, h	1, 2
S. Heidelberg	B	$\underline{1}$, 4, [5], 12	r	1, 2
S. Abortusovis	B	4, 12	c	1, 6
S. Abortusequi	B	4, 12	-	e, n, x
S. Typhisus	C_1	6, 7	c	1, 5
S. Choleraesuis	C_1	6, 7	c	1, 5
S. Montevideo	C_1	6, 7, $\underline{14}$	g, m, [p], s	-
S. Oranienburg	C_1	6, 7	m, t	-
S. Newport	C_2	6, 8	e, h	1, 2
S. Bovismorbificans	C_2	6, 8	r	1, 5
S. Kentucky	C_3	8, $\underline{20}$	i	z_6
S. Typhi	D_1	9, 12, [Vi]	d	-
S. Enteritidis	D_1	$\underline{1}$, 9, 12	g, m	[1, 7]
S. Dublin	D_1	$\underline{1}$, 9, 12, [Vi]	g, p	-
S. Gallinarum	D_1	$\underline{1}$, 9, 12	-	-
S. Pullorum	D_1	9, 12	-	-
S. Anatum	E_1	3, 10	e, h	1, 6
S. Newington	E_2	3, $\underline{15}$	e, h	1, 6
S. Senftenberg	E_4	1, 3, 19	g, [s], t	-
S. Worthongton	G_2	$\underline{1}$, 13, 23	z	i, w

[ ] = antigen may be present or absent, $\underline{1}$ = O factor whose presence is due to phage conversion (Adapted from Quinn *et al*., 1994).

2.1.4. Physiology and effects of environmental factors

2.1.4.1. Growth

Salmonellae organisms are typical of other Gram-negative bacteria in being able to grow on a large number of culture media and produce visible colonies well within 24 hours at about 37^0C in aerobic and anaerobic conditions. They are generally unable to ferment lactose, sucrose, or salicin, although glucose and certain other monosaccharides are fermented, with the production of gas (Old, 1990; Jay, 2000). Salmonellae actively grow within a wide temperature range ($\leq 54^0$C) and also exhibit psychrotrophic properties, as reflected in the ability to grow in foods stored at 2 to 4^OC (D'Aoust, 1997). The pH for optimum growth is around neutrality, with values above 9.5 and below 4.0 being bactericidal. For best growth the salmonellae require pH between 6.5 and 8.2 (D'Aoust, 1997; Jay, 2000). Water activity of less than 0.94 does not support the growth of salmonellae. They are generally inhibited in the presence of 3 to 4 % NaCl. The parameters of pH, water activity (a_w) or salt concentration, nutrient content and temperature of the microenvironment are all interrelated and can exert profound effects on the growth kinetics of *Salmonella* species (D'Aoust, 1997; Jay, 2000).

2.1.4.2. Resistance and survival properties in the environment

Compared with other Gram-negative rods, salmonellae are relatively resistant to various environmental factors. The survival of salmonellae for prolonged periods of time in foods stored at freezer and ambient temperatures is well documented. The viability of salmonellae in dry foods stored at $\geq 25^O$C decreases with increasing storage temperature and with increasing moisture content (WHO, 1988; D'Aoust, 1989). Although the low a_w in dry foods is not conducive to bacterial growth, the condition does promote survival of salmonellae for prolonged periods of storage at ambient and elevated temperatures. The resistance of *Salmonella* to heating depends on medium in which they are kept; greater heat resistance observed in salmonellae grown in nutritionally rich media than of salmonellae grown in minimal media (Seifert, 1996; D'Aoust, 1997).

The bacterium is sensitive to heat and will not survive temperatures above 70^oC. They are killed rapidly by autoclaving at 120^OC. At lower water activities, heat resistance may increase. Salmonellae have been shown to be resistant to drying even for years, especially in dried faeces, dust, and other dry materials such as feeds and certain foods. Prolonged survival

in water and soil has also been described (D'Aoust, 1997; Acha and Szyfres, 2001). The usual disinfectants (creolin 3%, chalk milk 5%, caustic soda 2%) inactivate the pathogens in a few minutes. Salmonellae are quite sensitive to beta and gamma irradiation (WHO, 1988; Seifert, 1996).

2.1.5. Biochemical characteristics

Salmonellae are chemoorganotrophic with an ability to metabolize nutrients by the respiratory and fermentative pathways. The organisms grow optimally at 37°C and catabolize D-glucose and other carbohydrates such as maltose, mannitol and sorbitol, with the production of acids and gas; failure to ferment sucrose, salicin and adonitol; failure to produce indole, to hydrolyze urea or to deaminate phenylalanine and a positive methyl-red reaction and a negative Voges-Proskauer reaction. Salmonellae are oxidase negative, catalase positive, grow on citrate as the sole carbon source, and generally produce hydrogen sulfide, decarboxylate lysine and ornithine (D'Aoust, 1989; Old, 1990; Bhatia and Ichhpujani, 1994; Quinn *et al.*, 1994).

2.1.6. Typing of *Salmonella*

Typing of *Salmonella* has been performed for decades. It can be used to differentiate the bacteria beyond species level. The essence of the use of typing methods is to be able to compare isolates, and to allocate strains with identical typing results into the same group (Olsen *et al.*, 1993). Phage typing, biotyping, drug resistance and plasmid profile analysis may be used to identify isolates beyond the level of serovars and are useful for epidemiologic studies (Clarke and Gyles, 1993).

2.1.6.1. Serotyping

While salmonellae are initially detected by their biochemical characteristics, groups and serotypes are identified by antigenic analysis. The classification of these organisms by antigenic analysis is based on the Kauffman and White scheme (Jay, 2000). Classification by this scheme makes use of both somatic and flagellar antigens (Jawetz *et al.,* 1995; Jay, 2000). In this classification, serovars are placed in groups designated A, B, C, and so on, according to similarities in content of one or more O antigens. For further classification, the flagellar or

H antigens are employed (Quinn *et al*, 1994; Popoff and Le Minor, 1997). Serotyping is a routine procedure for *Salmonella*, and typing sera for common serovars are commercially available in many countries. In the case of uncommon serovars, no further subdivision may be necessary, but those commonly encountered serotyping alone is not sufficiently discriminatory for epidemiological investigation. A means of subdivision is therefore required for common serotypes (Varnam and Evans, 1991).

2.1.6.2. Phage typing

Phage typing is based on the sensitivity of a particular isolate to a series of bacteriophages at appropriate dilutions (Quinn *et al*., 1994). 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 phage types (PT) (Olsen *et al*., 1993). Phage typing has been used successfully with many serovars in addition to *S.* Typhi. It has not been possible to develop phage typing schemes which are sufficiently discriminatory for some serovars. Acquisition of a drug resistance plasmid may change the phagovar of *Salmonella* (Varnam and Evans, 1991).

There is a high degree of correlation between phage type and epidemic source. Phage typing is presently one of the most sensitive epidemiological markers for the differentiation of Vi containing organisms. However, the technique is not without limitations. Some strains can not be phage typed because they have no Vi antigens, possess an inherent resistance to lytic phages, or fail to be recognised by existing grouping phages (D'Aoust, 1989; Old, 1990).

2.1.6.3. Biotyping

Biotyping has been widely used to clarify the biochemical heterogeneity among *Salmonella* species and to segregate serovars into serofermentative groups (D'Aoust, 1989). Subdividing common *Salmonella* serotypes according to their biochemical characters is some times of value in epidemiological investigations. In many serotypes there are few biochemical tests in which significant numbers of strains behave differently and so the numbers of identifiable biotypes are small. But, when available, biotyping is a useful adjunct to phage typing for it can subdivide a large group of untypable strains or members of common phage types (Old, 1990).

2.1.6.4. Genetic and molecular typing methods

Genetic typing methods are becoming used increasingly in epidemiological investigations. Of the available methods, plasmid profiling has been most widely used (Varnam and Evans, 1991). Most wild type strains of salmonellae carry plasmids differing in size and number. The plasmid may be electrophoresed in agarose gels and separated, on the basis of molecular size, to give a plasmid profile (Old, 1990; Olsen *et al*., 1993; Bhatia and Ichhpujani, 1994; Kariuki *et al*., 1999).

Plasmid typing is particularly useful in the epidemiology of exotic serotypes which appear from time to time and for which classical typing methods are not available (Old, 1990; Olsen *et al*., 1993). With some serovars plasmid profiling is most effectively used as an adjunct to phage typing, rather than for primary profiling (Varnam and Evans, 1991). Plasmid-profile analysis, however, is not always helpful and some times the information derived from the profile is limited. The demonstration that several strains harbor an indistinguishable plasmid can not always be taken as evidence of their relatedness (Old, 1990; Olsen *et al*., 1993). The technique facilitated differentiation of strains with identical antibiotic resistance patterns into epidemiologically related groups (D'Aoust, 1989; Kariuki *et al*., 1999). Studies on the characterization of epidemic strains of salmonellae by plasmid analysis, phage typing, antibiotic susceptibility, and biotyping underlined the equivalence of plasmid analysis and phage typing techniques and deficiencies in the other diagnostic methods. The limited value of antibiotic resistance patterns stems from the possibility that identical patterns in test strains could arise from different, none epidemiologically related R-plasmid factors (D'Aoust, 1989; Olsen *et al*., 1993; Bhatia and Ichhpujani, 1994).

2.1.7. Antimicrobial resistance

Until 1960 nearly all salmonellae were sensitive to a wide range of antimicrobial agents, but since 1962 plasmid-mediated resistance has appeared in them worldwide. The relative importance of antibiotic resistance, and the serotypes in which it develops, differs from country to country (Old, 1990; Kariuki *et al*., 1999; Acha and Szyfres, 2001).

Multiple-drug resistance is common in *Salmonella* and there is evidence that the use of antimicrobial drugs in animal feeds has contributed to selection for drug resistance *Salmonella*. The patterns of drug resistance of *Salmonella* isolates from animals vary with the

patterns of drug use in animal production (Clarke and Gyles, 1993). Current global trends depicting sustained increases in the number of antibiotic resistant *Salmonella* strains in humans and farm animals are most disquieting. The liberal administration of antimicrobial agents in hospitals and other treatment centers has led to the emergence and persistence of resistance strains (D'Aoust, 1997).

Food animals are now a major source of antimicrobial-resistant *Salmonella* infections in man. The correlation between the use of certain antibiotics as feed supplements and the increase in antibiotic resistance in bacteria appears to be well established. Multiple resistant salmonellae in foods of animal origin are more likely to cause infection in people who are already receiving antibiotic therapy (WHO, 1988; D'Aoust, 1997; Acha and Szyfres, 2001). Many *Salmonella* isolates are resistant to streptomycin, tetracycline, and sulphonamides; a moderately high percentage are resistance to ampicillin, kanamycine, neomycin and chloramphenicol; and only a low percentage are resistant to the fluoroquinolones, third generation cephalosporins, gentamicin, or ampicillin or ticarcillin combined with a beta lactamase inhibitor. Some serovars have remained relatively drug susceptible, but *S.* Typhimurium is commonly resistant to several drugs (Clarke and Gyles, 1993).

2.2. 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). Salmonellosis can affect all species of domestic animals including sheep and goats. Young, debilitated and parturient animals are most susceptible to clinical disease. Man is highly susceptible to infection, either by direct contact with infected animals or through their products. Salmonellosis is most prevalent in areas of intensive animal husbandry. Under conditions of extensive range husbandry the disease is most likely to manifest itself when the animals are stressed (Sewell and Brocklesby, 1990).

2.2.1. Prevalence of *Salmonella* in sheep and goats

The results of epidemiological investigations from many countries indicate that *Salmonella* infection in sheep and goats are prevalent. Generally the occurrence of *Salmonella* in sheep and goats in various countries varies from 1 to 51.5% (D'Aoust, 1989). In a number of countries, survey data from abattoirs and field investigations showed that salmonellae are common in sheep and goats. The prevalence in most of these countries is quiet high, Table 2.

Salmonellosis outbreaks with a high morbidity and mortality are not unknown. In goats, a morbidity rate of 50% and mortality rate of 38% were reported in transported feral goats in Australia (McOrist and Miller, 1981) and in sheep, a mortality rate of 50% in lambs was recorded in New Zealand (Hemmingsen *et al*., 1982). In infected flocks up to 20% of lambs or kids could die during the first 10 days of life (Leondidis *et al*., 1984).

Abortion is common in the naturally occurring disease of salmonellosis in sheep and goats. In Scotland, abortion rate ranging from 0.3 to 30% in sheep from different farms were reported during the period 1974 to 1981 (Linklater, 1983). The proportion of abortion in sheep and goats in Greece ranged from 10-60% (Leondidis *et al*., 1984). In India salmonellae were found in 1.3% of aborted does and 3.6% of aborted ewes (Verma *et al*., 1998).

Table 2. Prevalence of *Salmonella* in sheep and goats in different countries

Country	No. examined		Prevalence (%)		Reference
	Sheep	Goats	Sheep	Goats	
Australia	50	-	8.2	-	Mcaulliffe *et al.* (1978)
	25	-	4	-	Moo *et al.* (1980)
	100	-	43	-	Samuel *et al.* (1981)
	-	30	-	43	McOrist and Miller (1981)
New Zealand	2027	-	4.7	-	Kane (1979)
India	1659	-	2.2	-	Kapur *et al.* (1982)
	812	683	3.1	3.8	Kumar *et al.* (1973)
	-	100	-	12	Arora (1978)
	-	200	-	12	Das *et al.* (1990)
Siri Lanka	-	322	-	1.2	Subasinghe and Ramakrishna-swamy (1983)
Iran	500	-	2.6	-	Tadjbakhch *et al.* (1992)
Saudi Arabia	280	27	14.3	18.5	Nabbut and Al-Nakhli (1982)
	172	16	18.6	18.8	Nabbut *et al.* (1982)
Spain	30	-	10	-	Sierra *et al.* (1995)
Greece	62	-	8.1	-	Pateraki *et al.* (1975)
Italy	104	40	1.9	2.5	Cortesi *et al.* (1984)
Yugoslavia	-	100	-	13	Singh and Arora (1981)
Russia	NA	-	13.1	-	Kotova *et al.* (1988)
Argentina	100	-	2	-	Terzolo *et al.* (1979)
	-	200	-	6.5	Cortinez and Guzman (1987)
Senegal	1108	1018	4.7	3.6	Doutre and Boche (1976)
Nigeria	-	102	-	4.9	Falade (1976)
	200	200	4.0	9.5	Adesiyun *et al.* (1988)
	-	215	-	7.4	Uzoukwu and Mohan (1990)
Algeria	268	405	2.2	0.2	Mered *et al.* (1977)
Egypt	100	137	14	2.2	Abde-Ghani *et al.* (1987)
Zambia	NA	NA	1	2.3	Sharma *et al.* (1996)
Somalia	100	147	5	2	Andreani *et al.* (1979)
Ethiopia	47	60	6.4	16.7	Woldemariam (2003)

NA=Not available

2.2.2. Sources of infection and mode of transmission

Salmonellae are carried in the intestinal tracts and associated organs of most farm and wild animals. They are spread by direct or indirect means. Infected animals are the source of the organisms which they excrete and infect other animals directly or indirectly by contamination of the environment, primarily feed and water supplies. Ingestion of infected feed and water or contact with contaminated excretions is the main ways of infection (Baird-parker, 1990; Radostits *et al.,* 1994). Although *Salmonella* may survive for long periods in the environment, it is the carrier state that provides the major source of infection for animals and human. Thus, Persistence of infection in animals and in the environment is important epidemiological features of salmonellosis (Clarke and Gyles, 1993; Radostits *et al.,* 1994).

The mode of *Salmonella* transmission is complicated and a great number of animals, birds and reptiles are responsible for the maintenance of the chains of infection (Fig.1). A farm animal may be infected from various sources, including feeding stuffs, birds, bedding, flies, rodents, sewage, soil, and water. During transportation to slaughter, there may be cross contamination of animals since stress during transportation will often increase the excretion of *Salmonella* in the faeces. Crowding and prolonged lairage in abattoir pens predisposes animals to infection and surface contamination. There are opportunities for contamination at many of the stages during the slaughter, dressing, and preparation of raw meats. These are very important sources of *Salmonella* in human food chain. Several reports have stressed the impact of poorly disinfected knives and other slaughtering equipment and poor hygiene among plant personnel on carcass contamination (Smeltzer *et al*., 1980; Smeltzer and Thomas, 1981; D'Aoust, 1989; Baird-parker, 1990).

Transmission of *Salmonella* in sheep and goats is most commonly by the faecal oral route. The organism spreads geographically by the migration of excreting carrier animals and by the transportation of contaminated feed and water. The introduction of unrecognized carrier animals into susceptible population of sheep and goats is of major importance in the propagation and outbreak of salmonellosis. Cross species infection can also occur as a source of infection for sheep and goats (Kimberling, 1988; Smith and Sherman, 1994). Fleece laden in faecal material has been identified as an important source of carcass contamination. Wide spread contamination in rendered animal products and their incorporation into finished feeds ensure a continuous recycling of infection within the animal food chain (D'Auoust, 1989).

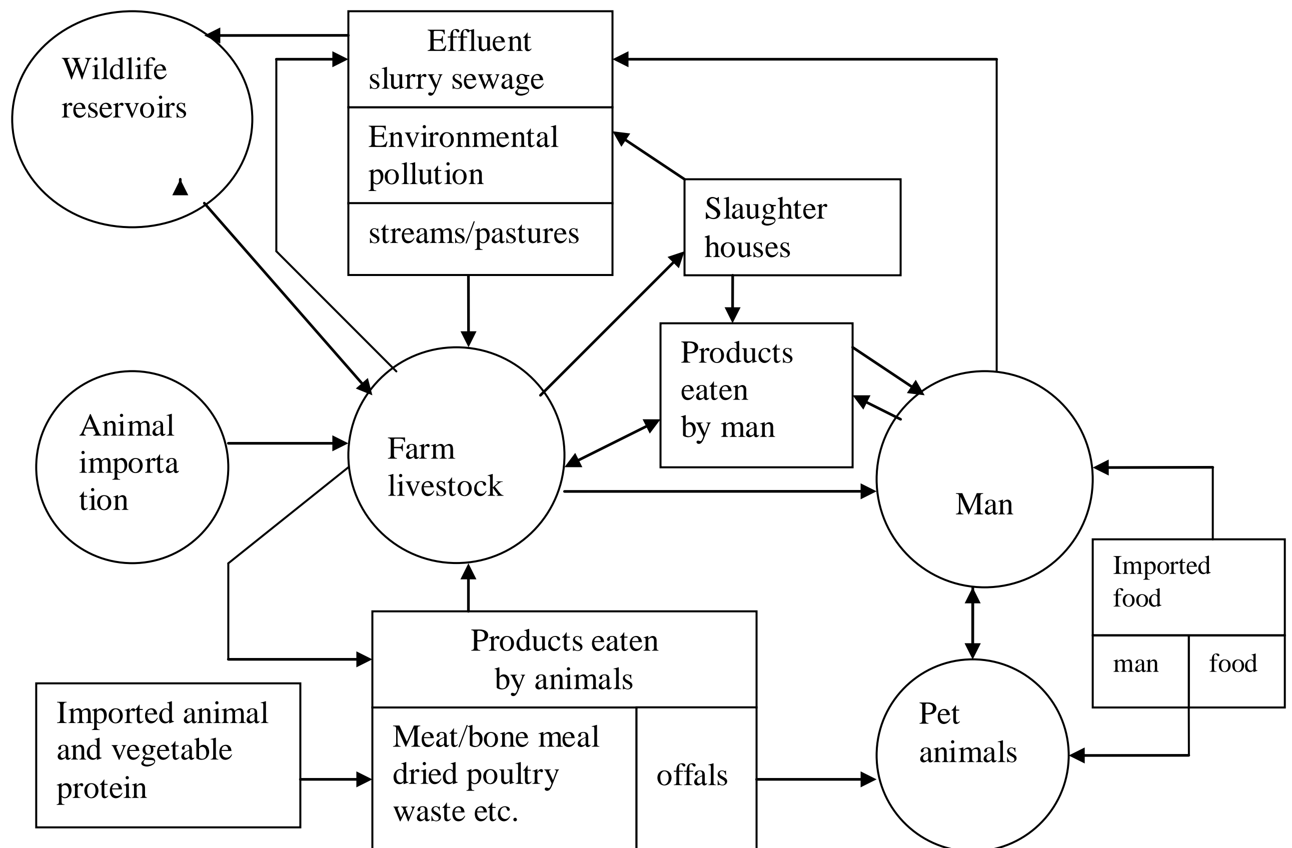


Fig. 1. *Salmonella* cycling and recycling (adapted from WHO, 1988).

2.2.3. Predisposing factors to clinical salmonellosis

The occurrence and out come of salmonellosis in sheep and goats can be influenced by a number of factors (Kimberling, 1988; Smith and Sherman, 1994). Except in the newborn, infection with *Salmonella* is not a single cause of salmonellosis. The response to infection with *Salmonella* varies depending on the size of the challenge dose, the immunological status of the animal, itself dependent on the colostrums intake in neonates, previous exposure to the infection and exposure to stressors, particularly in older animals (Radostits *et al.,* 1994). It is generally accepted that the intervention of some precipitating factors such as prolonged transport, intercurrent disease, impaired immunity, anaesthesia and surgery, dosing with antibiotics or anthelmintics, acute deprivation of feed and water, inclement weather or parturition is usually necessary to cause the disease in sheep and goats (Kimberling, 1988; Radostits *et al.,* 1994; Smith and Sherman, 1994).

Intensification of husbandry in all species is recognized as a factor contributing significantly to an increase in the new infection rate. Any significant change in management of the herd or group of animals can precipitate the onset of clinical salmonellosis, if the infection pre-exists in those animals. Intensive pasture utilization can result in contamination of pastures by the faeces of infected animals. Ingestion of this contaminated pasture leads to infection. Infection can be introduced into housed animals by infected domestic animals. Housed animals generally are more susceptible to infection from purchased feeds containing contaminated animal by products than are pastured animals, which are again more susceptible to animal product-based fertilizers. Introduction of the infection to a farm through contaminated feedstuffs, carrier animals, infected clothing of visitors and causal workers are the obvious ways (Radostits *et al.*, 1994).

2.3. Pathogenesis

Transmission of salmonellae is usually by the faecal oral route but infection via mucous membranes of the conjunctivae or upper respiratory tract is suspected. Salmonellae are often pathogenic to humans or animals when acquired by oral route. Salmonellae need to colonize the distal small intestine or colon to initiate enteric disease. Volatile organic acids produced by the indigenous normal anaerobic flora inhibit the growth of salmonellae and the normal flora usually blocks access to attachment sites required by the *Salmonella* species. Disruption of the normal intestinal flora by factors such as antibiotic therapy, diet, and water deprivation increases the host's susceptibility to infection. Reduced peristalsis, stress due to transportation and over crowding also predispose to the colonization of the intestine by salmonellae (Quinn *et al.,* 1994; Bhatia and Ichhpujani, 1994).

After ingestion, the pathogens rapidly grow and multiply in the rumen and pass into the small intestine. Marked enteritis develops most likely as a result of release of endotoxin from disintegrated organisms. Diarrhoea results atleast in part from the inflammatory enteritis, but the organisms may also elaborate enterotoxins that have a hyper secretory effect on villous epithelial cells leading to accelerated fluid and electrolyte loss from the gut (Kimberling, 1988; Smith and Sherman, 1994). In the small intestine, virulent *Salmonella* organisms penetrate mucous membranes. On invasion of the lymphatic, the bacteria enter Payer's patches, mesenteric lymph nodes and finally the systemic blood, which transport them to all organs. Colonization of the organism may occur in liver, gall bladder, spleen and mesenteric

lymph nodes. Death results from shock, septicaemia, endotoxemia, dehydration, and acidosis. Of the surviving sheep and goats, some become carriers and excretors of the organisms for variable periods. Animals experiencing infection may produce detectable antibodies against flagellar and somatic antigens of salmonellae (Kimberling, 1988; Smith and Sherman, 1994)

The invasive strains that produce septicemia are able to escape destruction by the host and to multiply within the macrophages of the liver and spleen as well as intravascularly. Destruction within the blood stream is prevented by O-repeat units of the lipopolysaccharide. It is thought that they may mask determinants on the bacterial cell surface that would normally bind complement and activate it by means of the alternative pathway. This would reduce the chances of chemotaxis, opsonisation and phagocytosis. Any salmonellae in non-immune animals that are phagocytosed tend to survive within the phagocyte. Siderophores, which remove iron from iron binding proteins of the host, are secreted by these invasive salmonellae. Multiplication of the organism in the body leads to a severe endotoxaemia (Quinn *et al*., 1994).

2.4. Salmonellosis in Sheep and Goats

Sheep and goat salmonellosis is an acute contagious disease, characterised by gastroenteritis, diarrhoea, septicaemia, metritis, abortion, and recovery of carriers. On a world wide basis, the diarrhoeal and septicaemia forms of clinical salmonellosis is commonly caused by *S.* Typhimurium, *S.* Bovismorbificans, *S.* Havana and others with *S.* Typhimurium being the most frequent pathogen (Kimberling, 1988; Smith and Sherman, 1994). Abortion may occur as a sequel to septicaemia with any *Salmonella* serotypes. *Salmonella* Typhimurium, *S.* Abortusovis, *S.* Montevideo, and *S.* Dublin are the usual causative serotypes of abortion in sheep and goats. These species may also cause enteric salmonellosis. These bacteria commonly reside in the alimentary tracts of carrier animals and contaminated feed and water (Kimberling, 1988; Radostits *et al.,* 1994; Smith and Sherman, 1994).

The disease occurs in all breeds, sex and ages of both sheep and goats, however, young and pregnant sheep and goats are most susceptible (Kimberling, 1988; Smith and Sherman, 1994; OIÉ, 2000). Because of universal distribution and high incidence within infected flocks, the disease has major economic importance to the entire sheep and goat industry. Economic

losses results from abortions, stillbirths, deaths and damaged wool (fiber) and from the cost of expensive treatment, prevention, extra labor, and disposal of dead sheep and goats. Additional losses come from incapacitation, discomfort, and treatment of infected people (Kimberling, 1988; Smith and Sherman, 1994; Radostits *et al.,* 1994).

In animals which survive the disease, localization of salmonellae organism occurs in gastrointestinal tract, mesenteric lymph nodes, liver, spleen and the gall bladder. In healthy adults there may be no clinical illness when infection first occurs but there may be localization in abdominal viscera. In either instance the animals become chronic carriers and discharge salmonellae intermittently (Radostits *et al.,* 1994). Numerous *Salmonella* serotypes have been isolated from the faeces of healthy goats and sheep and from viscera at slaughterhouses around the world (Kumar *et al*., 1973; Nabbut and Al-Nakhli, 1982; Smith and Sherman, 1994). This suggests that the carrier state is common in sheep and goats and there is considerable concern about the zoonotic potential of salmonellae contamination of sheep and goats meat in countries where it is widely consumed acting as a source of infection for man (Kumar *et al*., 1973; Nabbut and Al-Nakhli, 1982; Smith and Sherman, 1994).

2. 5. Diagnostic Methods for the Detection of *Salmonella*

The diagnosis of salmonellosis presents a considerable difficulty in the living animal largely because of the variety of clinical syndromes which may occur and the variations in necropsy findings. Mostly because of the non-specific clinical symptoms and necropsy findings, a presumptive diagnosis has to be confirmed by the isolation of the organism either from tissues collected aseptically at necropsy or from faeces, rectal swabs or environmental samples (Radostits *et al*., 1994; OIÉ, 2000).

2.5.1. Bacteriological examinations

Samples for bacteriological tests are collected as aseptically as possible and before any antibiotic treatment has commenced. Samples are collected preferably during the acute phase of the disease or as soon as possible after death (OIÉ, 2000). In the live animal, confirmation of clinical salmonellosis is performed by the culture of rectal swabs or preferably, freshly

voided faeces. The organisms may be isolated by blood culture and some time from the milk. At necropsy, the isolation of *Salmonella* from tissues and intestinal contents usually presents few problems, but care must be taken in the interpretation of the findings. The isolation of *Salmonella* should be correlated with clinical signs and pathological lesions in order to determine the significance of the isolation (Wray and Davis, 2000). The isolation and subsequent identification of *Salmonella* is dependent not only on the quality of samples but also on the culture medium and growth characteristics of the serovars, particularly those adapted to the host species (OIÉ, 2000).

2.5.1.1. Conventional cultural isolation techniques

According to the International Organization for Standardization (ISO 6579, 1998) it is customary to use three stage processes: pre-enrichment, selective enrichment and selective plating to isolate *Salmonella*.

2.5.1.1.1. Pre-enrichment

Pre-enrichment allows the resuscitation and multiplication of sub-lethally damaged *Salmonella* cells (Blackburn, 1993). Non-selective media such as buffered peptone water and lactose broth are most widely used for resuscitation; buffered peptone water being recommended for routine purposes. The need for resuscitation is now widely accepted for all types of samples and not merely those which have been dried or frozen (Varnam and Evans, 1991).

2.5.1.1.2. Selective enrichment

Selective enrichment helps to increase the ratio of *Salmonella* to competitor organisms. Many types of inhibitors have been proposed for the selective enrichment of *Salmonella,* the most widely used of which bile, tetrathionate, selenite and dyes are including brilliant green and malachite green. Various formulations of selenite and tetrathionate broths have been widely used, although in recent years there has been increasing use of the malachite green based Rappaport-Vassilliadis (RV) broth (Varnam and Evans, 1991; Blackburn, 1993).

2.5.1.1.3. Selective plating

Plating on selective agar media enables the recognition of *Salmonella* colonies while suppressing the growth of the back ground microflora. A wide range of media has been devised for selective plating. Selective plating media for *Salmonella* all contain a diagnostic system to permit differentiation of the organisms from non-*Salmonella.* This is commonly based on the inability of most salmonellas to ferment lactose and, in some cases, other carbohydrates such as sucrose and salicin. Bile containing media often employ a second diagnostic system based on the ability of *Salmonella* to produce hydrogen sulphide. Where competition from other bacteria is insignificant, a general-purpose medium such as MacConkey agars may be used. In many cases, greater selectivity is required and it is necessary to use a medium devised specially for *Salmonella*, such as brilliant green agar, *Salmonella-Shigella* agar, xylose lysine desoxycholate agar and Rambach agar (Varnam and Evans, 1991; Blackburn, 1993; Quinn *et al*., 1994).

2.5.2. Biochemical tests

Colonies characteristic for *Salmonella* on the selective/indicator media are inoculated singly onto a triple sugar iron (TSI) agar slope and lysine decarboxylase broth. The typical reactions for *Salmonella* in TSI agar is a red (alkaline) slant, yellow (acid) butt and superimposed (black) H_2S production (R/Y/ H_2S^+). Salmonellae typically produce an alkaline (purple) reaction in the tube of lysine decarboxylase broth. If the reaction in TSI agar and lysine decarboxylase broth is equivocal, further biochemical tests such as indole (negative), methylred (positive), citrate (positive), urease (negative), Voges-Proskauer test (negative) and others should be carried out or an identification system used such as API 20E (analytab products) or Enterotubes (Rohe Diagnostica) (Doyle and Cliver, 1990; Quinn *et al*., 1994).

2.5.3. Serological tests

Serological tests have been developed for the diagnosis of *Salmonella* infection. They are used to identify unknown cultures with known sera and may also be used to determine antibody titers in patients with unknown illness (Jawetz *et al.,* 1995; OIÉ, 2000). Slide agglutination test is one of the serological techniques used for the diagnosis of *Salmonella.* An agglutination reaction occurs when antibody reacts with particular antigen and cross-links

surface antigen determinants. A drop of polyvalent antiserum is added to a suspension of bacteria in saline. A control of the bacterial suspension without antiserum is also used to eliminate autoagglutination. After the addition of antiserum the slide is rocked gently and the result is read within 3 minutes. Clumped bacteria indicate a positive reaction. Serological identification is made more precise by typing with monospecific serums (Quinn *et al*., 1994).

2.6. Public Health Importance of Salmonellosis

Salmonellosis is globally one of the major foods borne infection in humans. It is a real or potential problem in all areas of the world (WHO, 1988). It occurs both in sporadic cases and outbreaks affecting a family or several hundreds or thousands of people in a population. Foodborne salmonellosis in humans continues to be a major public health problem in many countries (Bean *et al*., 1990). In developed countries it is an important foodborne disease and accounts for the majority of all outbreaks of such disease cases, hospitalizations and deaths, where the causative agent is identified. It is difficult to evaluate the situation of this disease in developing countries because of lack of epidemiologic surveillance data but epidemic outbreaks are known to occur (Acha and Szyfres, 2001).

Animals are the reservoir of zoonotic *Salmonella.* Practically any food of animal origin can be a source of infection for man. Meat, milk, pork, poultry, eggs and egg products are the commonest human foods of animal origin which may be contaminated at source (Fig. 2) and cause infection in man (D'Aoust, 1997; Acha and Szyfres, 2001). *Salmonella* live in the intestinal tracts of humans and other animals, including birds. Salmonellae are usually transmitted to humans by eating food contaminated with animal faeces. Contaminated foods are often of animal origin but all foods including vegetables may become contaminated. Many raw foods of animal origin are frequently contaminated but fortunately thorough cooking kills *Salmonella.* Food may also become contaminated by the unwashed hands of an infected food handler, who forgot to wash his or her hands with soap after contact with faeces (D'Aoust, 1997).

Although some of the *Salmonella* infections cause disease, the majority probably lead to sub clinical cases resulting in a healthy carrier state with intermittent excretion of *Salmonella* in the faeces. Whether a human develops disease following ingestion of salmonellae depends on the dose of organisms, the serotypes of *salmonella* and upon specific and non-specific

immunological factors (Quinn *et al*., 1994). The serovars involved in salmonellosis vary geographically but frequently includes *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, *S.* Agona, *S.* Newport, *S.* Infantis, *S.* Panama, *S.* Saintpoul and *S.* Weltevreden. While some serovars maintain their dominant role over many years, others emerge and decrease over time (ICMSF, 1996). A rapid international trade in agricultural, aquaculture and manufactured food products has greatly facilitated the introduction of new *Salmonella* serovars within the geographical boundaries of importing countries (D'Aoust, 1994). Where for decades *S.* Typhimurium predominated, *S.* Enteritidis has now become the dominant serovar responsible for human illness. Incidence of *S* .Virchow has also increased considerably since 1991 and a slow progression of *S.* Hadar was noted especially in 1994 and 1995 in many European countries (Uyttendaele, 1998).

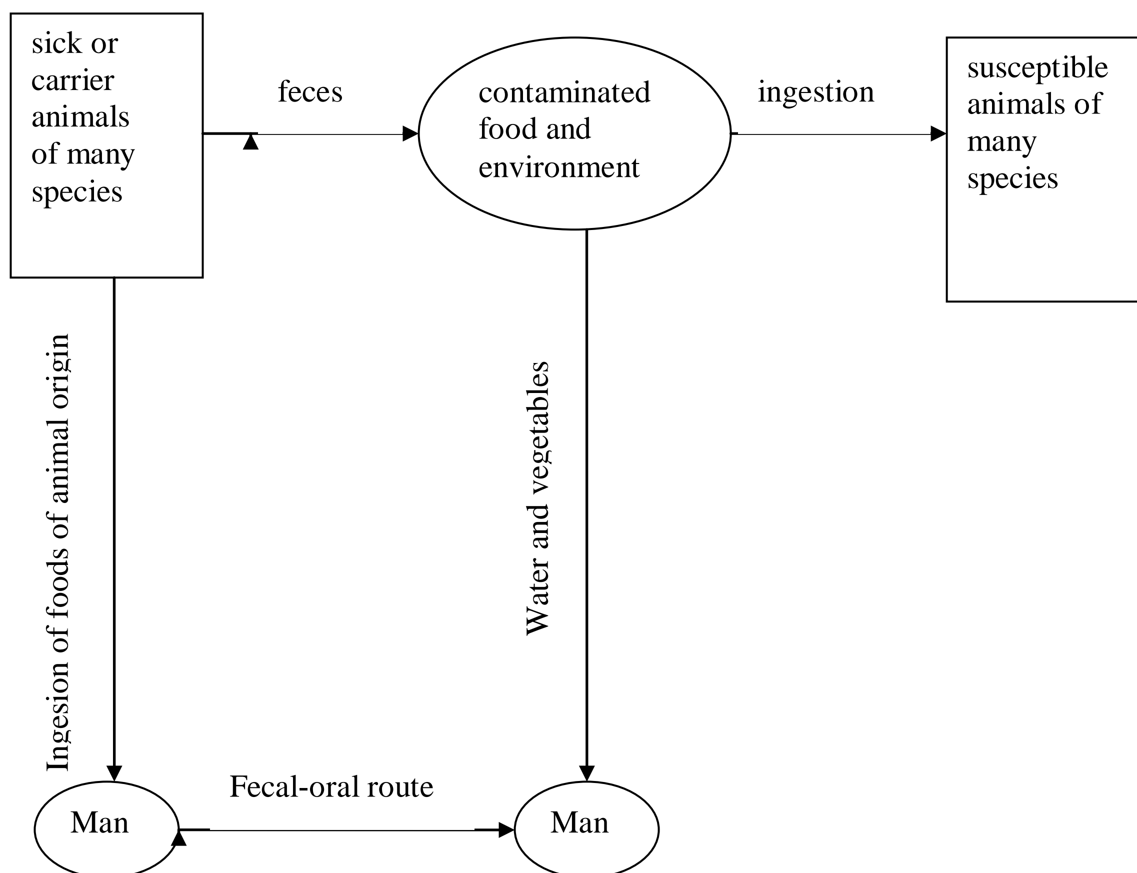


Fig.2. Mode of transmission of *Salmonella* (except *Salmonella* Typhi and the Paratyphoid serotypes) (source: Acha and Szyfres, 2001)

Human salmonellosis is widespread in young children, in elderly citizens frequently afflicted with underling chronic diseases, and in immunosuppressed individuals. Infants one year of

age or younger are particularly susceptible to infection. Transmission of the infectious agent occurs primarily between humans, from various animals and environment (D'Aoust, 1989). Changes in eating habits, mass catering and increased international movement of foods and food ingredients have certainly contributed to the observed increase in foodborne salmonellosis outbreaks (D'Aoust, 1989; D'Aoust, 1994).

2.7. Economic Importance of Salmonellosis

Salmonellosis is one of the significant causes of economic loss in farm animals because of the cost of clinical disease which include deaths, diagnosis and treatment of clinical cases, diagnosis laboratory costs, the cost of cleaning and disinfection, and the cost of control and prevention. The loss incurred by the livestock producers includes feed efficiency, and reduced weight gains or deaths because of salmonellosis (Radostits *et al.*, 1994). Human foodborne salmonellosis constitutes a major health problem in many countries. Moreover the costs associated with salmonellosis could be considerable (Persons and Jendteg, 1992). Financial costs are not only associated with investigation, treatment and prevention of human illness but may affect the chain of production (Sockett, 1991).

2.8. Status of Salmonellosis in Ethiopia

The studies conducted in Ethiopia indicated the presence of *Salmonella* in animals, humans, foodstuffs and environment. Salmonellae were isolated from man, chicken, cattle, camels, sheep, goats and food (Pegram *et al.,* 1981; Mache *et al.*, 1997; Molomo, 1998; Molla *et al.,* 1999a; Nyeleti *et al.*, 2000; Tibaijuku, 2003; Woldemariam, 2003). In a study conducted by Pegram *et al*. (1981), 27 *Salmonella* serotypes were isolated from samples of animal origin in different parts of the country. This study indicated that salmonellosis is an important disease of dromedary calves and poultry in Ethiopia. Molomo (1998) recorded 19.8% average mortality rate caused by the *Salmonella* in commercial poultry farms. Molla *et al.* (1999a) reported 19.0% contamination rate of retailed food (chicken and minced beef) by *Salmonella* in Addis Ababa. In a study carried out in selected site of Addis Ababa isolation rate of 10.6%, 19.6%, 9.8%, 11.9%, from cattle faeces and mesenteric lymph nodes, abdominal and

diaphragmatic muscle respectively and 6%, from abattoir personnel were recorded (Nyeleti *et al.*, 2000). In and around Debre-Zeit, 3.1% of diaphragmatic muscles, 2.8% of abdominal muscles, 3.1% of faecal and 4.5% of mesenteric lymph node contamination rate of cattle were reported (Alemayehu *et al.*, 2003). A prevalence of 42% from raw '*kitfo*' (minced meat) samples in Addis Ababa was reported by Tegegne and Ashenafi (1998). An overall contamination rate of 17.9% was also found in retail chicken meat and offals from supermarket of Addis Ababa (Tbaijuka *et al.,* 2003).

In sheep and goats, Woldemariam (2003) reported a 6.4% and 16.7% prevalence of salmonellosis respectively at Elfora Debre Zeit abattoir. Ejeta *et al*. (2004) reported a 14.1% *Salmonella* contamination rate of mutton collected from Addis Ababa supermarkets. According to Woldemariam (2003), out of the 642 samples from apparently healthy sheep (n=282) and goats (n=360) examined for *Salmonella,* 33 *Salmonella* isolates consisting of 9 different serotypes were identified. Ten (3.5%) of apparently healthy slaughtered sheep and 23 (6.4%) of goat samples were found to be contaminated with *Salmonella*. *Salmonella* were detected in 2 (1.8%), 3 (8.4%), 5 (4.6%), and 7 (6.5%) of each 107 samples of spleen, faeces, liver, and mesenteric lymph nodes, respectively. The serotype identified were *S.* Infantis (45*%), S.* Butanta*n* (24.2%), *S.* Braenderu*p*, *S.* Kingabwa (each 6%), *S.* Zanzibar, *S.* Kottbus, *S.* Anatum, *S.* Typhimurium and *S.* Hadar (each 3%). In samples collected from sheep only 4 serotypes were detected (*S.* Kottbus, *S.* Infantis, *S.* Braenderup and *S.* Kingabwa) whereas in goats all the 9 serotypes were identified. *Salmonella* Infantis and *S.* Butantan were the most common serotypes isolated from samples originated from apparently healthy slaughtered sheep and goats. Ejeta *et al*. (2004) also isolated five different serotypes (*S.* Infantis (25%), *S.* Braenderup (42%), *S.* Anatum (8.3%), *S.* Bovismorbificans (17%), and *S.* I: 47, z_4, z_{23}) from mutton; the predominant serotype being *S.* Braenderup followed by *S.* Infantis.

In another study *Salmonella* isolation rate of 4.5% was recorded from stool specimens of diarrhoeal out-patients from various hospitals and clinics in Addis Ababa (Ashenafi and Gedebou, 1985). Furthermore Mache (2002) reported a prevalence of 15.4% from paediatric diarrhoeal out-patients in Jimma hospital and Jimma health centre.

Different workers have tested the antimicrobial resistance of *Salmonella* isolates from animals and human beings. According to Molla *et al.* (1999b), the frequency of antimicrobial resistance among *Salmonella* serotypes isolated from chicken and minced beef in Addis Ababa was high. From 39 isolates, 34 (87.2%) were resistant to one or more antibiotics.

Ninety percent of the isolates from minced beef samples were found to be resistant to one of or more antimicrobials tested. The rate of resistance of *Salmonella* isolates from gizzard, liver and heart was 87.5%, 71.4% and 75.0%, respectively. The antimicrobials to which *Salmonella* isolates were most often fully resistant include nitrofurantoin (48.7%), furazolidone (48.7%) and streptomycin (46.2%). Nyeleti *et al*. (2000), found 100% susceptible *Salmonella* isolates of cattle and minced beef against 17 drugs tested which is difficult to discuss. In a study undertaken by Alemayehu *et al*. (2003), resistance to three or more antimicrobials was detected in 52% (13/25) of the *Salmonella* isolates. However, of the five different *Salmonella* serovars detected, only strains of *S*. Mishmarhaemek and *S*. Typhimurium were found to be resistant. All *Salmonella* isolates from sheep and goats (Woldemariam, 2003) were susceptible to 24 antimicrobials tested (Amikacin, amoxicillin/clavulanic acid, ampicillin, apramycin, carbadox, cephalothin, ceftriaxone, ceftiofur, cefoxitin, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, kanamycin, nalidixic acid, neomycin, nitrofurantoin, spectinomycin, trimethoprim, streptomycin, sulfisoxazole, tetracycline, tobramycin and sulfamethoxazole/trimethoprim). In another study, among 59 *Salmonella* strains isolated from diarrhoeal stools of paediatric outpatients in Jimma hospital and Jimma health centre, 59.3% were resistant to tetracycline and ampicillin, 47.5% to cephalothin, 40.7% to trimethoprime-sulfamethoxazole, 35.6% to chloramphenicol, and less than 25.4% were resistant to other drugs (Mache, 2002).

The surveys mentioned above which mainly concentrated in Addis Ababa and its surrounding indicated directly or indirectly that the role of animals in the epidemiology of salmonellosis is very important. Furthermore, the habit in this country to consume, raw meat as a traditional food poses a high risk of acquiring Salmonellosis as well as other zoonotic diseases (Molla *et al.,* 1999a; Molla *et al.,* 2003a).

2.9. Prevention and Control

The control of *Salmonella* in meat animals and derived products is a very challenging task because of the complexity and interdependence of various aspects of animal husbandry, slaughtering, and processing (D'Aoust, 1989). National and international control policies have been set up with the aim of reducing salmonellosis in animals in order to reduce outbreaks of human foodborne infections (WHO, 1988). Coordinated control efforts from the farm level to

the food processor and to retail outlets, together with comprehensive educational programs for the consumers and food handlers, are indispensable if we are to record marked reduction in the prevalence of *Salmonella* in foods and related incidence of human disease (D'Aoust, 1989). The application of salmonellosis control measures throughout the food-chain-live animals, animal products, processing and final food preparation varies greatly from country to country, depending on the animal species involved and also on the condition of import, export, and tourism (WHO, 1988).

The application of the hazard analysis critical control point (HACCP) system throughout the food chain to identify the best places (critical control points) to eliminate or control growth or contamination with *Salmonella* is a pre-requisite for the effective and economic control of human and animal salmonellosis (Baird-parker, 1990). The principle of control includes prevention of introduction and limitation of spread within a herd (Aiello and Mays, 1998). Every effort must be made to prevent introduction of carrier animals. Animals should be purchased directly only from farms known to be free of the disease. Ensuring that feeds of supplies are free of salmonellae depends on the integrity of the source (Aiello and Mays, 1998). In order to limit the spread within a herd, in all outbreaks, the following procedures should be implemented: Carrier animals should be identified and either culled or isolated and treated vigorously. The prophylactic use of antibiotics in feed or water supplies may be considered. Movements of animals around the farm should be restricted to limit the infection to the smallest group. Random mixing of animals should be avoided. Feed and water supplies must be protected from faecal contamination. Contaminated buildings must be vigorously cleaned. Contaminated materials must be disposed carefully. All persons should be aware of the hazards of working with infected animals and the importance of personal hygiene (Aiello and Mays, 1998).

Transportation of animals to abattoirs or markets should be done under conditions that minimize stress, and in vehicles designed to be cleaned and disinfected between uses. Hygiene design and operation of holding areas is necessary to limit animal to animal spread of salmonellosis. Hygienic abattoir practices are extremely important with respect to the contamination of meat with *Salmonella*. Antemortem and post-mortem inspection are important for removal of diseased animals from the food chain, although the vast majority of animals carrying *Salmonella* in their intestinal tracts do not show any visual signs of infection (Baird-parker, 1990).

3. MATERIALS AND METHODS

3. 1. Study Areas

The study was undertaken at Addis Ababa public abattoir in Addis Ababa and Modjo modern export abattoir at Modjo, Ethiopia. Addis Ababa is the capital city and administration centre for the Federal Democratic Republic of Ethiopia. It lies in the central highlands of Ethiopia at an altitude of 2500 meters above sea level and has an estimated human population of about 3 million. The average annual temperature and rain fall are 21°C and 1800 mm respectively. Addis Ababa has a relative humidity varying between 70% to 80% during the rainy season and 40% to 50% during the dry season (NMSA, 1999).

Modjo town is the center of Lume district in Eastern Shewa administrative zone of Oromia Regional State. It located 73 kilometers South East of Addis Ababa at an altitude of 1777 meters above sea level. The average maximum and minimum temperature are 28°C and 18°C respectively.

The microbiological analysis of the samples was undertaken at the Microbiology Laboratory of Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

3. 2. Study Population

The study population consisted of apparently healthy slaughtered sheep and goats at Addis Ababa public and Modjo modern export abattoirs and apparently healthy abattoir personnel of the two abattoirs. The sheep and goats slaughtered at Addis Ababa abattoir were originated from different parts of the country, mainly from North Shewa of Amhara and Oromia region, Jimma, Gojjam, Wollo, East Shewa, Wellega, West Shewa, Afar, Somali, Borana and Arsi. The sheep and goats are usually brought to the Addis Ababa market individually or in flocks on foot or by truck. The animals are purchased by restaurant owners and taken to Addis Ababa abattoir for slaughter. The animals are slaughtered after antemortem examination with in 1 to 6 hours of their arrival to the abattoir. In this abattoir an average of 250 sheep, 75 goats

and 700 heads of cattle are slaughtered daily. In addition about 50 swines are slaughtered per week. The abattoir has separate slaughtering sections for small ruminants, swine and cattle.

The sheep and goats slaughtered at Modjo modern export abattoir originated mostly from Bale, Metehara, Borana, Arbaminch, Measo and Babile. The animals are usually brought to the abattoir by truck in flocks and they are transported for an approximate distance of 500 km from their origin to the abattoir. The animals get rest for 24 to 72 hours and slaughtered after antemortem inspection by authorized veterinarians. In the abattoir only sheep and goats are slaughtered for export mainly to Arabian countries. On average about 51 sheep and 509 goats are slaughtered daily.

The Addis Ababa public abattoir and Modjo modern export abattoir have about 700 and 77 manpower respectively. These include both office and production line workers. On production line, there are about 420 individuals in Addis Ababa public abattoir and 53 workers in Modjo modern export abattoir.

3.3. Study Design

A cross-sectional study was undertaken in Addis Ababa public and modjo modern export abattoirs from September 2003 to February 2004. On each sampling day, usually once a week, 12 animals were randomly selected and sampled from amongst apparently healthy slaughtered sheep and goats. Faecal, mesenteric lymph nodes, liver, spleen, abdominal and diaphragmatic muscle samples were aseptically taken from each apparently healthy slaughtered sheep and goats in separate sample containers. Stool samples were also collected from apparently healthy abattoir personnel in collaboration with the medical personnel in the abattoirs clinics.

Each sample was collected aseptically and transported in ice box packed with ice to the microbiology laboratory of the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit for analysis. The samples were stored at freezer temperature before processing for no longer than 24 hours. All samples were processed and analyzed separately.

3.4. Sampling

3.4.1. Sample size

The sample size required for the study was determined depending on the expected prevalence of salmonellosis and the desired absolute precision according to Thrusfield (1995) by the following formula.

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

Where:

n = required sample size

Pexp = expected prevalence

d = desired absolute precision

Using a 95% confidence level, 5% absolute precision and 7% expected prevalence for goat and 7% for sheep (Woldemariam (2003) reported a prevalence of 6.4% in sheep at Elfora Debre Zeit abattoir), the desired sample size was 100 sheep and 100 goats. Even though, the desire sample size was as indicated here in reality 104 sheep and 100 goats were sampled during the study period. The human sample size was 117 voluntary and accessible apparently healthy personnel of both abattoirs.

3.4.2. Sampling procedures

On each sampling day, 12 selected sheep and goats (6 types of samples from each animal=72 samples) were sampled. From each selected animal sufficient amount of faecal and mesenteric lymph nodes, about 100 gm of liver, spleen, diaphragm and abdominal muscles (M. Obliquus abdominis, M. Transversus abdominis) samples were collected separately in sterile containers. All the samples were collected aseptically with sterile forceps and scalpel blades. As soon as the abdomen of the animal was opened, the intestine with the mesenteric lymph nodes were separated from the rest of gastrointestinal tract and kept in a separate clean container until the other tissue samples collection from the same animal have been completed. Approximately 100 gm of spleen, liver, abdominal muscle and diaphragm muscle samples were collected and put in sterile aluminium foil. Following this, the mesenteric lymph nodes and faecal samples

were collected and put in a large size test tube and sterile universal bottle respectively. Human stool samples were collected in sterile universal bottles from voluntary apparently healthy abattoir personnel in collaboration with medical personnel at the clinics. Each sample was labeled legibly and accompanied by necessary identifying information, which include date of sampling, type of sample, species of animal from which the sample was obtained and identification code. The samples were then transported to the laboratory for analysis. A total of 1224 different kinds of samples were collected from sheep and goats and 117 stool samples from voluntary and accessible abattoir personnel.

3.5. Isolation and Identification of *Salmonella*

Salmonellae were identified and isolated according to the techniques recommended by the International Organization for Standardization (ISO 6579, 1998). The detection of *Salmonella* necessitates different successive stages (Fig. 3). The bacteriological media used in different stage were prepared according to the manufacturer's recommendations as indicated in appendix I.

3.5.1. Pre-enrichment

The samples were thawed at room temperature for 3 to 4 hours before processing. The required amount (25 gm) of each sample of mesenteric lymph nodes, liver, spleen, abdominal and diaphragm muscles was weighted and cut into smaller fine pieces with sterile scalpel blades. Each sample was put in a sterile stomacher bag and 225 ml of buffered peptone water (BPW; Sifin, Berlin, Germany) was added, homogenized using a stomacher laboratory blender (Colworth stomacher 400, London) at high speed for two minutes and incubated for 16 to 20 hours at 37°C. Faecal and stool samples and samples smaller than 25 gm were pre-enriched in buffered peptone water in a ratio of 1 gm of the sample to 9 ml of buffered peptone water.

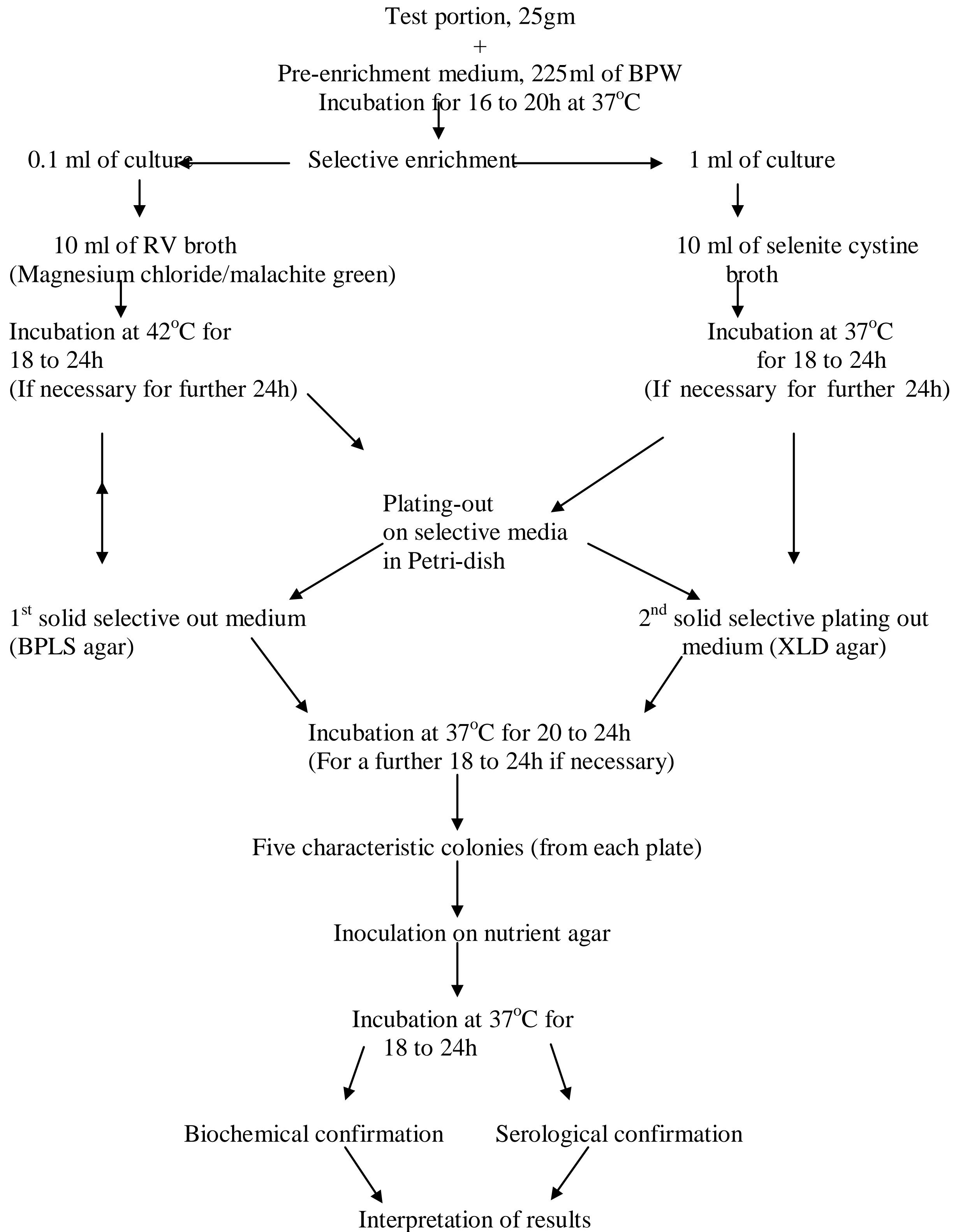


Fig. 3. Flow diagram showing the horizontal method for the detection of *Salmonella* (ISO 6579, 1998)

3.5.2. Selective enrichment

Magnesium chloride malachite green broth acc. to Rappaport Vassilliadis (RV) (Sifin, Berlin, Germany) and selenite cystine (SC; Merck, Darmstadt, Germany) broth media were used for selective enrichment. After incubation, 1 ml of the pre enrichment broth was transferred aseptically into 10 ml of selenite cystine and incubated at $37^{O}C$ for 18 to 24 hours. Another 0.1 ml of the same pre-enrichment broth culture was transferred into 10 ml of Rappaport Vassilliadis broth and incubated at $42^{O}C$ for 18 to 24 hours.

3.5.3. Selective platting and identification

Brilliant green-phenol red agar acc. to Edel and Kampelmacher (BPLS; Sifin, Berlin, Germany) and xylose lysine desoxycholate agar (XLD; Sifin, Berlin, Germany) agar plates were used for selective plating. A loopful of inoculum from Rappaport-Vassilliadis and selenite cystine broths culture were streaked onto BPLS agar plates and XLD agar plates. The inoculated plates were incubated at $37^{O}C$ for 24 to 48 hours. After incubation, the plates were examined for the presence of *Salmonella* colonies. Typical colonies of *Salmonella* growing on BPLS agar give an alkaline reaction and have red colonies while on XLD medium they produce hydrogen sulphide and have red colonies with a black (H_2S) centre (Quinn *et al.,* 1994; ISO 6579, 1998).

3.5.4. Confirmation

For confirmation, five presumptive *Salmonella* colonies were selected from every selective plating media. If the suspected colonies on each plate are fewer than five, all the colonies were selected. The selected colonies were streaked onto the surface of nutrient agar (Oxoid, Hampshire, England) plates, in a manner which allowed well isolated colonies to develop. The inoculated plates were incubated at $37^{O}C$ for 18 to 24 hours. The pure cultures on nutrient agar were used for biochemical and serological confirmation.

3.5.4.1. Biochemical tests

Suspected *Salmonella* colonies were tested biochemically according to ISO 6579 (1998) using triple sugar iron (TSI) agar, urease, lysine decarboxylase, citrate, and methyl red and indole tests.

Triple sugar iron agar test: The TSI agar (Merck, Darmstadt, Germany) tube was inoculated in the middle of the agar to within 5mm from the bottom of the tube with a straight inoculating wire. On the withdrawal of the straight wire, the entire slant was streaked (right to the top) and incubated at $37^{O}C$ for 16 to 24 hours. The typical reactions for most salmonellae are red slant, yellow butt with gas production and superimposed black color with hydrogen sulphide production (Quinn *et al.,* 1994; ISO 6579, 1998).

Citrate utilization test: Simmon citrate agar (Difco, Detroit, USA) slope surface was streaked with the suspected *Salmonella* colonies and incubated at $37^{O}C$ for 18 to 24 hours. Typical reactions for citrate utilization and positivity for *Salmonella* is declared by the change of the medium from green to blue color (Quinn *et al.,* 1994).

Lysine decarboxylase test: The lysine decarboxylase broth (Difco, Detroit, USA) was inoculated with the suspected *Salmonella* colonies just below the surface of the liquid medium and incubated for 18 to 24 hours at $37^{O}C$. Purple color after incubation indicates a positive reaction for *Salmonella* whereas a yellow color indicates a negative reaction (Quinn *et al.,* 1994; ISO 6579, 1998).

Urease test: Heavy inoculum of the presumptive *Salmonella* colonies was inoculated into the urea broth (Merck, Darmstadt, Germany) tube and incubated at the $37^{O}C$ for about 48 hours. *Salmonella* organism does not split urea and this was noticed by yellow color of the broth while a red color indicates a positive reaction for urease test (Quinn *et al.,* 1994).

Methyl red (MR) test: MR-VP broth (Methyl red Voges-Proskauer broth) (Merck, Darmstadt, Germany) tube was inoculated with suspected *Salmonella* colonies and incubated at $37^{O}C$ for 48 hours. After incubation about 5 drops of the methyl red indicator solution (Merck, Darmstadt, Germany) was added to the broth culture. The change of the medium color from orange to red indicates a positive reaction. Salmonellae are positive for MR test (Quinn *et al.,* 1994).

Indole test: The presumptive *Salmonella* colonies were inoculated into trypton water (Merck, Darmstadt, Germany) broth and incubated at $37^{O}C$ for about 48 hours. After incubation 1 ml of Kovacs reagent (Merck, Darmstadt, Germany) was added into the broth culture. The formation of a red ring indicates a positive reaction whereas a yellow-brown ring indicates a negative reaction for indole test. *Salmonella* is negative for indole test (Quinn *et al.,* 1994; ISO 6579, 1998).

For further confirmation, the suspected *Salmonella* colonies which became positive for batteries of biochemical tests were streaked onto the surface of Rambach agar (Merck, Darmstadt, Germany) plates and incubated at $37^{O}C$ for 18 to 24 hours. Characteristic colonies which appeared red on Rambach agar were considered as *Salmonella.*

3.5.4.2. Serological tests

Colonies that exhibit typical reactions for the different biochemical tests were further screened for the presence of *Salmonella* antigens by slide agglutination test using polyspecific test reagent "anti-*Salmonella* I" (A-E) (Sifin, Berlin, Germany) and polyspecific test serum "anti-*Salmonella* II" (Sifin, Berlin, Germany). First, the bacteria of suspected colonies were tested with the polyspecific test reagent "anti-*Salmonella* I". The "anti-*Salmonella* II" test was only applied, if the test with the test reagent "anti-*salmonella* I" did not yield a positive result. Both tests were performed following the instructions of the producer.

The isolates which became positive for *Salmonella* were cultured on tryptic soy agar (Difco, Sparks, USA) and sent to Health Canada, Office International des Épizooties (OIÉ) Reference Laboratory for Salmonellosis 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 Épizooties (OIÉ) 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 Épizooties (OIÉ) 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 (AT). Strains which did not react with any of the typing phages were considered untypable (UT). The phage typing scheme and phages for *Salmonella* Typhimurium, developed by Callow (1959) and further extended by Anderson (1964) and Anderson and Colleagues (1977) were obtained from the Central Public Health Laboratory, Colindale, London, United Kingdom. *Salmonella* Heidelberg strains were phage typed with the phages isolated and the phage typing scheme developed at the Laboratory Center for Disease control in Ottawa, Ontario (Khakhria *et al.*, 1997). *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 3. 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.

Table 3. Antimicrobials and concentrations used to test susceptibility of *Salmonella* isolates

Antimicrobial	Abbreviations	Antimicrobial break point concentrations[a]	
		Susceptible at	Resistant at
		≤ 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	FLO[d]	ND	16[h]
Gentamicin	GEN	ND	16
Kanamycin	KAN	ND	64
Nalidixic acid	NAL	ND	32
Neomycin	NEO[d]	ND	16[e]
Nitrofurantoin	NIT	ND	64[i]
Spectinomycin	SPT[d]	ND	64[e]
Streptomycin	STR[d]	ND	32[e]
Sulfisoxazole	SUL	ND	521
Sulfamethoxazole/trimethoprim	SXT	ND	76/4
Tetracycline	TET	ND	16
Tobramycin	TOB	ND	8
Trimethoprim	TMP	ND	16

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

[b]ND –not done.

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

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

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

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

[g]A 0.125 μg/ml of ciprofloxacin concentration determines reduced sensitivity to ciprofloxacin

[h] Strains were considered to be resistant to florfenicol at the level of 16 μg/ml

[i] 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 collected were entered and managed in MS Excel soft ware programs. Graph was drawn in MS Excel programs. An intercooled Stata 7 soft ware (Stat Corporation, 2001) statistical program was employed for the data analysis.

Descriptive statistics were used to understand the nature and the characteristics of the data, to see the rate of *Salmonella* detection in each sample type and distribution of *Salmonella* isolation. Prevalence estimation of *Salmonella* in apparently healthy slaughtered sheep and goats and abattoir personnel was determined using standard formulae (that is the number of positive animals or humans was divided by the total number of animals or humans examined to obtain proportion of infection). The exact binomial confidence limits of prevalence were determined using the exact binomial confidence interval.

Chi square (χ^2) test was employed to assess if there was a statistically significant difference in the rate of *Salmonella* isolation in sheep between abattoirs. Fisher's exact test was used to determine whether there were significant differences in *Salmonella* isolation rate in goats between abattoirs and among different sample types examined in both species. For this analysis P-value <0.05 were considered significant whereas P-value >0.05 considered non significant.

Logistic regression was employed to see the association between factors and *Salmonella* prevalence. The strength of the association was measured using odds ratio. Factors with odds ratio greater than one were considered as risk factors and those with odds ratio less than one were protective factor. A risk factor or protective factor was considered significant if the confidence interval didn't include one. Test of differences of deviances was also used to see the interaction between factors. In this case P-value <0.05 were considered significant whereas P-value >0.05 considered non significant

4. RESULTS

A cross-sectional study on *Salmonella* in apparently healthy slaughtered sheep and goats and apparently healthy abattoir personnel was conducted at Addis Ababa public and Modjo modern export abattoirs between September 2003 and February 2004. The objectives of the study were to determine the prevalence and distribution of *Salmonella* in sheep and goats, identify and characterize the prevalent *Salmonella* serotypes, determine the infection level in abattoir personnel and to determine the antimicrobial resistance of *Salmonella* isolated from apparently healthy slaughtered sheep and goats and abattoir personnel.

4.1. Prevalence of *Salmonella*

A total of 104 sheep, 100 goats and 117 abattoir personnel were sampled and examined for the presence of *Salmonella* in their body tissue, faeces and stool samples. *Salmonella* was isolated from 15 (7.4%) animals of which 80% and 20% were sheep and goats respectively, Table 4.

Table 4. Occurrence of *Salmonella* in apparently healthy slaughtered sheep and goats at Addis Ababa and Modjo abattoirs

Abattoir	Species	No. examined	No. positive	Prevalence (%)	[95% Confidence Interval] (%)
Addis Ababa	Ovine	46	6	13.0	4.9-26.3
	Caprine	45	1	2.2	0.1-11.8
Modjo	Ovine	58	6	10.3	3.9-21.2
	Caprine	55	2	3.6	0.4-12.5
Addis Ababa + Modjo	Ovine	104	12	11.5	6.1-19.3
	Caprine	100	3	3.0	0.6-8.5
Total		**204**	**15**	**7.4**	**4.2-11.8**

The over all prevalence of *Salmonella* in apparently healthy slaughtered sheep from the two abattoirs was 11.5% (12/104). The prevalence at Addis Ababa public abattoir and Modjo modern export abattoir were 13.0% (6/46) and 10.3% (6/58) respectively, Table 4. Though, higher prevalence of *Salmonella* was found in apparently healthy slaughtered sheep at Addis

Ababa public abattoir (13%) than Modjo modern export abattoir (10.3%), there was no a statistically significant (P>0.05) difference in the prevalence between these two abattoirs.

Of the total 100 apparently healthy slaughtered goats examined from the two abattoirs, 3 goats (3%) were found *Salmonella* positive, Table 4. A prevalence of 2.2% (1/45) and 3.6% (2/55) was also found at Addis Ababa public abattoir and Modjo modern export abattoir respectively. No significant (P>0.05) difference was observed in the proportion of *Salmonella* isolation between the two abattoirs with respect to goats.

There was a significant (P<0.05) difference in the isolation of *Salmonella* between species (Table 5). Sheep was four times more likely to harbor *Salmonella* than goats (OR = 4.2 (95% CI 1.2-15.4)). There was no significant (P>0.05) difference in the proportion of *Salmonella* isolation between abattoirs. In addition, the interaction between species and abattoirs was not statistically significant (P>0.05), Table 5.

Salmonella was not isolated from any of the 117 human stool samples collected from Addis Ababa public (n=69) and Modjo modern export (n=48) abattoir personnel.

Table 5. Association of species and abattoirs with *Salmonella* isolation in sheep and goats

Variables	Df	Deviance	Resid. Df	Resid. Dev	P-value
Null			3	6.1926	
Abattoir	1	0.0277	2	6.1649	0.8678
Species	1	5.8435	1	0.3214	0.0156
Species * Abattoir interaction	1	0.3214	0	-1.665e-14	0.5708

4.2. Isolation of *Salmonella* from Different Sources

In this study a total of 1224 tissue and faecal samples were collected and examined from sheep (n=624) and goats (n=600) slaughtered at the two abattoirs. *Salmonella* was identified from 22 (1.8%) samples of which 81. 8% and 18.2% were from sheep and goats respectively (Table 6 and 7).

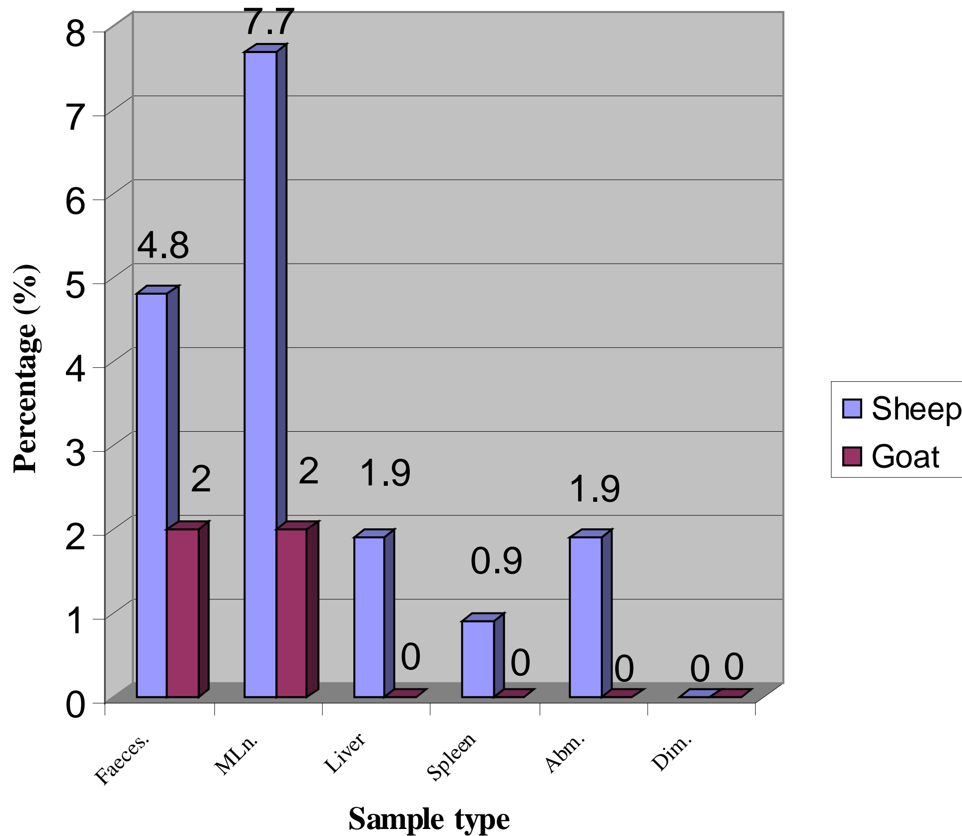
Table 6. Isolation of *Salmonella* in tissue and faecal samples of apparently healthy slaughtered sheep at Addis Ababa and Modjo abattoirs

Sample type	Number of samples from Addis Ababa abattoir		Modjo abattoir		Total	
	examined	positive (%)	examined	positive (%)	examined	positive (%)
Mesenteric lymph nodes	46	4 (8.7)	58	4 (6.9)	104	8 (7.7)
Feaces	46	3 (6.5)	58	2 (3.5)	104	5 (4.8)
Liver	46	1 (2.2)	58	1 (1.7)	104	2 (1.9)
Abdominal muscle	46	1 (2.2)	58	1 (1.7)	104	2 (1.9)
Spleen	46	1 (2.2)	58	0 (0.0)	104	1 (0.9)
Diaphragmatic muscle	46	0 (0.0)	58	0 (0.0)	104	0 (0.0)
Total	**276**	**10 (3.6)**	**348**	**8 (2.3)**	**624**	**18 (2.9)**

Of the total 624 samples examined from apparently healthy slaughtered sheep for human consumption, 18 (2.9%) were *Salmonella* positive (Table 6). Salmonellae were isolated from 4.8% of 104 faecal samples, 7.7% of 104 mesenteric lymph nodes samples, 1.9% of 104 liver samples and 1.9% of 104 abdominal muscle samples. Only one isolate was found from 104 samples of spleen and *Salmonella* was not detected from 104 diaphragmatic muscle samples. The proportion of positive samples therefore ranged from 0% in diaphragmatic muscle to 7.7% in mesenteric lymph nodes. The over all proportion of positive samples of fecal and mesenteric lymph nodes was higher than the proportion of positive liver, spleen, abdominal and diaphragmatic muscle samples (Fig. 4), However there was some variation between abattoirs (Table 6).

Twelve *Salmonella* positive sheep were detected from a total of 104 apparently healthy slaughtered sheep for human consumption (Table 4). Two sheep were identified as positive by faecal samples only, 4 sheep by mesenteric lymph nodes samples only and 2 animals by abdominal muscle samples only. The maximum number of isolates in one animal was 4 (Appendix II). The difference in the frequency of *Salmonella* isolation among faecal, mesenteric lymph nodes, liver, spleen, abdominal muscle and diaphragmatic muscle

specimens were statistically significant ($P<0.05$). However, there was no significant ($P>0.05$) difference in isolation of *Salmonella* in specimens of apparently healthy slaughtered sheep between the two abattoirs.



Abm. = Abdominal muscle, Dim. = Diaphragmatic muscle, MLn. = Mesenteric lymph nodes

Fig. 4. *Salmonella* isolation in different sample types

Four (0.7%) *Salmonella* positive samples were detected from a total of 600 samples from apparently healthy slaughtered goats for human consumption (Table 7). *Salmonella* was isolated from 2 (2%) faecal and 2 (2%) mesenteric lymph nodes samples. In other organs *Salmonella* was not detected. Of the total 100 apparently healthy slaughtered goats examined, 3 were found *Salmonella* positive (Table 4). In one of these, it was found in faeces only, while in another goat it was isolated from mesenteric lymph nodes and not from faeces. There was one goat in which *Salmonella* was isolated from both faeces and mesenteric lymph nodes (Appendix III).

In goats too, the number of positive specimens was higher for faeces and mesenteric lymph nodes samples than liver, spleen, abdominal and diaphragmatic muscle samples (Fig. 4), even though, the proportion of *Salmonella* isolation among these specimens was not significantly (P>0.05) different. Furthermore, there was no a significant (P>0.05) difference in the isolation rate of *Salmonella* from goats specimens between abattoirs.

Table 7. *Salmonella* isolation in tissue and faecal samples of apparently healthy slaughtered goats at Addis Ababa and Modjo abattoirs

	Number of samples from					
Sample type	Addis Ababa abattoir		Modjo abattoir		Total	
	examined	positive (%)	examined	positive (%)	examined	positive (%)
Feaces	45	1 (2.2)	55	1 (1.8)	100	2 (2.0)
Mesenteric lymph nodes	45	0 (0.0)	55	2 (3.6)	100	2 (2.0)
Liver	45	0 (0.0)	55	0 (0.0)	100	0 (0.0)
Spleen	45	0 (0.0)	55	0 (0.0)	100	0 (0.0)
Abdominal muscle	45	0 (0.0)	55	0 (0.0)	100	0 (0.0)
Diaphragmatic muscle	45	0 (0.0)	55	0 (0.0)	100	0 (0.0)
Total	**270**	**1 (0.4)**	**330**	**3 (0.9)**	**600**	**4 (0.7)**

4.3. *Salmonella* Serotypes Isolated

The serotypes isolated and the number of sheep and goats from which each organism was recovered, are shown in Table 8 and 9.

A total of 22 *Salmonella* isolates, consisting of 9 different serotypes were identified from sheep and goats. All the serotypes recovered during the study are listed in Table 8. Of the positive samples, the most common serogroups recovered were B (68.2%), followed by E_1 and G (each 9.1%) and others (13.6%), Table 9. The most prevalent serotypes isolated were *S.* Typhimurium (36.4%), followed by *S.* Heidelberg (18.2%), *S.* Reading, *S.* Give, and *S.* Poona (each 9.1%), Table 9.

The distribution of serotypes in abattoirs (Table 8) showed that *S*. Typhimurium (63.6%) and *S*. Reading (18.2%) were the two most dominant serotypes found at Addis Ababa public abattoir whereas *S*. Heidelberg (36.4%), *S*. Give (18.2%) and *S*. Poona (18.2%) were the dominant serotypes at Modjo modern export abattoir.

Table 8. Distribution of *Salmonella* serotypes by animal species and abattoir source

Serotype	Source and number of serotypes				Total		
	Addis Ababa abattoir		Modjo abattoir				
	sheep	goats	sheep	goats	sheep	goats	Total
S. Typhimurium	6	1	1	-	7	1	8
S. Typhimurium var. Copenhagen	1	-	-	-	1	-	1
S. Heidelberg	-	-	4	-	4	-	4
S. Reading	2	-	-	-	2	-	2
S. Give	-	-	2	-	2	-	2
S. Poona	-	-	-	2	-	2	2
S. Enteritidis	1	-	-	-	1	-	1
S. I: 6,7,14:-:enz15	-	-	-	1	-	1	1
S. Niederoderwitz	-	-	1	-	1	-	1
Total	**10**	**1**	**8**	**3**	**18**	**4**	**22**

In sheep among 18 *Salmonella* isolates, 7 different *Salmonella* serotypes were identified (Table 8 and 10). These were *S*. Typhimurium (38.9%) the prevalent one, *S*. Heidelberg (22.2%), *S*. Reading and *S*. Give (each 11.1%), *S*. Typhimurium var. Copenhagen, *S*. Enteritidis and *S*. Niederoderwitz (each 5.6%). The maximum isolates in one animal were four (in faeces, mesenteric lymph node, liver and spleen) consisting two serotypes (*S*. Typhimurium and *S*. Typhimurium var. Copenhagen). Two serotypes (*S*. Heidelberg and *S*. Typhimurium) were also detected from one sheep (Table 10). Three different serotypes were recovered from goats of which *S*. Poona (50.0%) was the dominant one followed by *S*. Typhimirium and *S*. I: 6, 7, 14:-:enz15 (each 25.0%). *Salmonella* Niederoderwitz (from sheep), *S*. Poona and *S*. I: 6, 7, 14:-:enz15 (from goats) were reported for the first time in Ethiopia.

Table 9. *Salmonella* serotypes isolated from sheep and goats

Serogroup	Serotype	No. isolated	%
B (O: 4)	*S.* Heidelberg	4	18.2
	S. Reading	2	9.1
	S. Typhimurium	8	36.4
	S. Typhimurium var. Copenhagen	1	4.6
D_1 (O: 9)	*S.* Enteritidis	1	4.6
E_1 (O: 3, 10)	*S.* Give	2	9.1
G (O: 13)	*S.* Poona	2	9.1
U (O: 43)	*S.* Niederoderwitz	1	4.6
Others	*S.* I: 6, 7, 14:-:enz15	1	4.6
Total		**22**	**100**

4.4. *Salmonella* Phage Types Identified

The results of phage typing indicated that all the eight *S.* Typhimurium isolates were phage typed into 5 different phage types. They were *S.* Typhimurium phage type 46 (37.5%), *S.* Typhimurium phage type 193 (25.0%), *S.* Typhimurium phage type 2, *S.* Typhimurium phage type 79, and *S.* Typhimurium phage type U285 (each 12.5%). Phage type 193 was found only in faeces, phage type 2, 79 and U285 in mesenteric lymph nodes and phage type 46 in liver, spleen and abdominal muscle. *Salmonella* Typhimurium var. Copenhagen phage type 104 and *S.* Enteritidis phage type 5a were also isolated in mesenteric lymph nodes and faeces respectively. All the identified phage types of *S.* Heidelberg were atypical and were isolated in faeces, mesenteric lymph nodes and abdominal muscles (Table 10). All the isolated phage types were detected in sheep. In goats only *S.* Typhimurium phage type 193 was isolated. Most of the isolated serotypes and phage types were found in mesenteric lymph nodes and faeces except *S.* Typhimurium phage type 46, *S.* Heidelberg phage type atypical and *S.* Give which were detected in tissues beyond mesenteric lymph nodes (Table 10).

Table 10. Distribution of *Salmonella* serotypes and phage types in various sample types of sheep and goats

Species	Animal code	Sample type	Serotype	Phage type
Ovine	S_{11}	Faeces	*S*. Typhimurium	193
		Mesenteric lymph node	*S*. Typhimurium var. Copenhagen	104
		Liver	*S*. Typhimurium	46
		Spleen	*S*. Typhimurium	46
	S_{13}	Mesenteric lymph node	*S*. Typhimurium	79
	S_{17}	Mesenteric lymph node	*S*. Typhimurium	2
	S_{30}	Faeces	*S*. Enteritids	5a
	S_{39}	Abdominal muscle	*S*. Typhimurium	46
	S_{46}	Faeces	*S*. Reading	-
		Mesenteric lymph node	*S*. Reading	-
	S_{48}	Mesenteric lymph node	*S*. Give	-
		Liver	*S*. Give	-
	S_{77}	Abdominal muscle	*S*. Heidelberg	Atypical
	S_{86}	Mesenteric lymph node	*S*. Heidelberg	Atypical
	S_{92}	Mesenteric lymph node	*S*. Niederoderwitz	-
	S_{93}	Faeces	*S*. Heidelberg	Atypical
	S_{94}	Faeces	*S*. Heidelberg	Atypical
		Mesenteric lymph node	*S*. Typhimurium	U285
Caprine	G_{37}	Faeces	*S*. Typhimurium	193
	G_{49}	Faeces	*S*. Poona	-
		Mesenteric lymph node	*S*. Poona	-
	G_{81}	Mesenteric lymph node	*S*. I: 6,7,14:-:enz1	-

4.5. Antimicrobial Resistance of *Salmonella* Isolates

A total of 22 *Salmonella* isolates recovered from sheep (n=18) and goats (n=4) were tested for resistance using 24 different antimicrobials (Table 3). Seven (31.8%) of the isolates were found to be resistant (Table 11). The level of antimicrobial resistance for sheep and goats isolates were 6 (33.3%) and 1 (25.0%) respectively. Of the 9 *Salmonella* serovars isolated,

only strains of *S*. Typhimurium and *S*. Reading were resistant. *Salmonella* Typhimurium phage type 46 and 193 were the resistant strain of *S*. Typhimurium (Table 11). All the resistant isolates were detected from Addis Ababa abattoir while isolates from Modjo abattoir were susceptible to all antimicrobials tested.

Table 11. Distribution of antimicrobial resistance of *Salmonella* isolates by source

Species	Sample type	Serotype	Phage type	Number tested	Number of strain resistant
Ovine	Faeces	*S*. Enteritidis	5a	1	0
		S. Typhimurium	193	1	1
	Faeces, MLn.*	*S*. Reading	-	2	2
	Faeces, MLn., Abm.	*S*. Heidelberg	Atypical	4	0
	MLn., liver	*S*. Give	-	2	0
	MLn.	*S*. Niederoderwitz	-	1	0
		S. Typhimurium var. Copenhagen	104	1	0
		S. Typhimurium	79	1	0
		S. Typhimurium	2	1	0
		S. Typhimurium	U285	1	0
	Liver, spleen, Abm.	*S*. Typhimurium	46	3	3
Caprine	Faeces	*S*. Typhimurium	193	1	1
	Faeces, MLn.	*S*. Poona	-	2	0
	MLn.	*S*. I: 6, 7, 14:- :enz15	-	1	0
Total				**22**	**7**

*MLn. =Mesenteric lymph nodes Abm. =Abdominal muscle

All isolates were susceptible to the antimicrobial effects of amikacin, apramycin, carbadox, ceftriaxone, ceftiofur, cefoxitin, ciprofloxacin, florfenicol, gentamicin, kanamycin, nalidix acid, neomycin, nitrofurantoin and tobramycin. Resistance to the remaining 10 antimicrobials varied between 4.6% and 18.2% (Table 12). Resistance to ampicillin, sulfisoxazole, streptomycin and cephalothin (each 18.2%) was common among the isolates and followed by tetracycline (13.6%), Table 12. *Salmonella* Typhimurium isolates showed resistance to ampicillin and cephalothin (each 50.0%), sulfisoxazole, streptomycin, sulfamethoxazol-

trimethoprim and trimethoprim (each 25.0%), amoxicillin/clavulanic acid, chloramphenicol, spectinomycin and tetracycline (each 12.5%). However, *S*. Reading isolates showed resistance to streptomycin, sulfisoxazole and tetracycline (each 100%), Table 12.

Table 12. Resistance of *Salmonella* isolates from sheep and goats by antimicrobial type

Antimicrobial	Number of resistant serotypes (%)		
	S. Typhimurium	*S*. Reading	All isolates
Ampicillin	4 (50.0)	0 (0.0)	4 (18.2)
Amoxicillin/clavulanic acid	1 (12.5)	0 (0.0)	1 (4.6)
Cephalothin	4 (50.0)	0 (0.0)	4 (18.2)
Chloramphenicol	1 (12.5)	0 (0.0)	1 (4.6)
Spectinomycin	1 (12.5)	0 (0.0)	1 (4.6)
Streptomycin	2 (25.0)	2 (100)	4 (18.2)
Sulfisoxazole	2 (25.0)	2 (100)	4 (18.2)
Sulfamethoxazol/trimethoprim	2 (25.0)	0 (0.0)	2 (9.1)
Tetracycline	1 (12.5)	2 (100)	3 (13.6)
Trimethoprim	2 (25.0)	0 (0.0)	2 (9.1)
Total	**5 (62.5)**	**2 (100)**	**7 (31.8)**

Multiple drug resistance (resistance to two or more drugs) was detected in 31.8% of all the isolates. All the resistant strains of *S*. Typhimurium and *S*. Reading showed multiple drug resistance (Table 13). Of the total *S*. Typhimurium (n=8) and *S*. Reading (n=2) isolates, 5 (62.5%) and 2 (100) respectively were resistant to multiple drugs. *Salmonella* Typhimurium PT 193 demonstrated multiple-drug resistances to up to 9 different antimicrobials (AmpAmcCefChlSptStrSulSxtTmp). Different antimicrobial resistance patterns were displayed among the resistant *Salmonella* strains. The common resistance pattern was to AmpCef in 3 (42.9%) of the resistant strains followed by resistance to StrSulTet observed in 2 (28.6%), Table 13 and 14.

Table 13. Mutlidrug resistance pattern of *Salmonella* isolates

Serotype	Number of strains			Antimicrobial resistance pattern
	Tested	Resistant (%)	Multiple resistant (%)	
S. Enteritidis	1	0 (0.0)	0 (0.0)	
S. Reading	2	2 (100)	2(100)	StrSulTet*
S. Heidelberg	4	0 (0.0)	0 (0.0)	
S. Give	2	0 (0.0)	0 (0.0)	
S. Niederoderwitz	1	0 (0.0)	0 (0.0)	
S. Typhimurium var. Copenhagen	1	0 (0.0)	0 (0.0)	
S. Typhimurium	8	5 (62.5)	5 (62.5):	
			3	AmpCef
			1	StrSulSxtTetTmp
			1	AmpAmcCefChl-SptStrSulSxtTmp
S. Poona	2	0 (0.0)	0 (0.0)	
S. I: 6, 7, 14:- :enz15	1	0 (0.0)	0 (0.0)	
Total	**22**	**7 (31.8)**	**7 (31.8)**	

*For key of abbreviations see Table 3.

From all *Salmonella* isolates tested, 15 (68.2%) were sensitive to all 24 antimicrobial drugs. Three (13.6%) *Salmonella* isolates were resistant to 2 antimicrobials, 2 (9.1%) to 3 antimicrobials, 1 (4.5%) to 5 antimicrobials and 1 (4.5%) to 9 antimicrobials (Table 14).

Table 14. Resistance of *Salmonella* isolates by number of antimicrobials

Resistance to no. of antimicrobials	Resistance pattern	No. of isolates (%)
Zero		15 (68.2)
Two	AmpCef*	3 (13.6)
Three	StrSulTet	2 (9.1)
Five	StrSulSxtTetTmp	1 (4.5)
Nine	AmpAmcCefChlSptStrSulSxtTmp	1 (4.5)

*For key of abbreviations see Table 3.

5. DISCUSSION

5.1. Prevalence

The isolation of *Salmonella* from various meat producing animals is not unusual. In the present study, salmonellae were isolated from 11.5% of the sheep and 3% of the goats investigated. The prevalence of *Salmonella* was high in sheep in contrast to goats. This difference was statistically significant (P<0.05). Furthermore, sheep was more than four times (OR = 4.2) more likely to harbor *Salmonella* than goats. This high variation in prevalence of *Salmonella* between sheep and goats might be due to differences in feeding behavior (sheep prefer to graze while goat to browse) and rearing area as well as management differences in the two species. However, it needs further investigation to identify clearly the factors which are responsible for the variation.

The proportion of *Salmonella* carrier rate in sheep reported in this study (11.5%) was high. The higher prevalence in sheep might be due to higher salmonellae carrier rate in the study population. In addition, the sheep involved in this study came from different parts of the country by different transport means and were usually held for 6 to 72 hours before slaughter. The close contact during the transport and holding time may account for the high prevalence of *Salmonella* when examined after slaughter (Hurd *et al.*, 2002). Furthermore, in this study multiple (n=6) samples were included from the same animal this could increase the likely hood of detecting a positive animal. This finding was in consistent with a report of 2 to 51.5% prevalence in sheep from different countries (D'Aoust, 1989), 13.1% from sheep carcasses in meat processing plants in Russia (Kotova *et al.*, 1988), 14% from slaughtered sheep in Egypt (Abdel-Ghani *et al.*, 1987), 14.3% prevalence from slaughtered sheep at Riyadh public abattoir in Saudi Arabia (Nabbut and Al-Nakhli, 1982) and 10% from freshly dressed carcasses in Spain (Sierra *et al.*, 1995).

Our results however, were higher than the previous findings (6.4%) of Woldemariam (2003) at Elfora Debre Zeit abattoir in Ethiopia. The difference in the prevalences reported could be due to differences in study period, study site (abattoirs), study population and number of samples examined between the two studies. It is also higher than the findings of 1% prevalence in Zambia (Sharma *et al.*, 1996), 3.1% from slaughtered sheep in India (Kumar et al., 1973), 4.0% from slaughtered sheep in Australia (Moo *et al.*, 1980), 8.1% from sheep

slaughtered at Athens abattoir in Greece (Paterkie *et al.*, 1975), 8.2% from exported live sheep in Australia (Mcauliffe *et al.*, 1978), 2% from healthy flock of sheep in Argentina (Terzolo *et al.*, 1979), 5% from slaughtered sheep in Somalia (Andreani *et al.,* 1979), 2.2% in Algeria (Mered *et al.*, 1977), 4% from slaughtered sheep in Zaria, Nigeria (Adesiyun *et al.*, 1988), 1.92% in apparently healthy slaughtered sheep in Italy (Cortesi *et al.*, 1984) and 2.6% in Iran (Tadjbakhch *et al.*, 1992). In majority of these studies, only one or two types of samples were examined and a higher percentage of infection would probably have been obtained if other organs had been examined. While this finding is much lower than that of Nabbut *et al.* (1982) who reported 18.6% isolation rate in Saudi Arabia and Samuel *et al.* (1981), who found 43% infection rate in Australia. The animals involved in those study were most likely had traveled and been held in the animal market for long periods before slaughter.

A higher prevalence of *Salmonella* was found from sheep slaughtered at Addis Ababa Abattoir (13.0%) than Modjo modern export abattoir (10.3%), though the difference was not statistically significant. This difference could be due to the difference in hygienic standard between the two abattoirs. Modjo modern export abattoir is more hygienic than Addis Ababa abattoir and is implementing HACCP principles and slaughtered animals are destined for export. This finding support the finding of Serra *et al.*(1995), who found higher prevalence of *Salmonella* infection in poor hygienic standard abattoir than hygienically better abattoirs.

The prevalence of *Salmonella* in apparently healthy slaughtered goats in this study was 3%. This demonstrated that the prevalence of *Salmonella* in goats was low. Indeed, the prevalence of infection in goats before they reached the abattoir was probably lower than shown in this survey as there was circumstantial evidence of lairage infection. This result fall in the range of 1 to 18.8% prevalence in goats from different countries (D'Aoust, 1989). This finding was also comparable to the findings of Cortesi *et al.,* (1984), who reported 2.5% prevalence in apparently healthy slaughtered goats in Naples, Italy, Abdel-Ghami *et al.* (1987) 2.2% from apparently healthy goats in Egypt, Doutre and Boche (1976) 3.6% from slaughtered goats in Dakar, Senegal, Sharma *et al.* (1996) 2.3% from goats samples in Zambia, Falade (1976) 4.9% from slaughtered goats at Kano abattoir in Nigeria and Kumar *et al.* (1973) 3.8% from slaughtered goats in India.

The present finding was much lower than the 16.7% prevalence reported from goats slaughtered at Elfora Debre Zeit abattoir in Ethiopia (Woldemariam, 2003). The low prevalence estimate in goats at Addis Ababa and Modjo abattoirs may be due to several

reasons. First, the proportion of *Salmonella* infected goat herds in the present study may be lower than in most of the areas represented in the previous studies. In the present study salmonellae were isolated from faeces and mesenteric lymph nodes samples only while contrary to this Woldemariam (2003) reported high isolation rate in meat cuts. This implies serious contamination could have occurred at Elfora Debre Zeit abattoir during slaughtering process. The contamination might occur through infected knives, abattoir equipment and abattoir personnel (Smeltzer and Thomas, 1981; Smeltzer *et al.*, 1980). This could be one reason for the high prevalence at Elfora Debre Zeit abattoir and the other could be due to study site (abattoirs) and study period differences of the two studies. In addition, animals to be slaughtered at Elfora Debre Zeit abattoir stayed in the collection site for a longer time and in the abattoir atleast for one week they were kept crowded which might have facilitated the transfer of infection among the animals. Holding of animals in lairages for more than two days, generally enhances the spread of salmonellae and other zooonotic agents (WHO, 1988). Furthermore, in Elfora Debre Zeit abattoir both large and small ruminants were slaughtered in the same room this might facilitate cross-contamination.

This finding was also lower than the 13% prevalence in goats meat reported in Yugoslavia (Singh and Arora, 1981), 18.5% from slaughtered goats in Riyadh, Saudi Arabia (Nabbut *et al.*, 1982), 12% from slaughtered goats in India (Arora, 1978; Das *et al.*, 1990), 7.4% in apparently healthy slaughtered goats in Nigeria (Uzoukwu and Mohan, 1990), 6.5% from slaughtered goats in Argentina (Cortinez and Guzman, 1987) and 9.5% from slaughtered goats in Zaria, Nigeria (Adesiyun *et al.*, 1988). However, the frequency of isolation of *Salmonella* obtained in the present study was higher than the 0.3% reported by Mered et al. (1977) from goats in Algeria, 0% by Wernery and Thimm (1978) from goats in Somalia and 1.2% by Subasinghe and Ramakrishnaswamy (1983) from slaughtered goats in Sri Lanka.

In this study *Salmonella* was not found from 117 stool samples of abattoir personnel of both Addis Ababa and Modjo modern export abattoirs. This finding is contrary to the 6% prevalence reported by Nyeleti *et al.* (2000) from stool samples of Addis Ababa abattoir personnel. The difference between the results of the previous and the present one could have been due to improvement of the health and hygienic conditions of the abattoir personnel. Moreover, there is abattoir personnel health check up program regularly and rarely in Modjo modern export abattoir and Addis Ababa abattoir respectively. Low contamination rate of *Salmonella* in sheep and goats meat cuts has been observed at both abattoirs. This could be explained by low isolation rate of *Salmonella* in abdominal muscles and no detection of

Salmonella in diaphragmatic muscles samples. The low isolation rate in meat cuts of sheep and goats indicate that the contamination of meat by abattoir personnel during the slaughtering process was minimal. The low contamination rate observed in both abattoirs could strengthen the finding of no carrier of *Salmonella* in abattoir personnel.

From a total 1224 sheep and goat samples examined 1.8% was infected with *Salmonella*. Only 0.7% of goat samples were positive compared with 2.9% of sheep samples and they contribute 18.2% and 81.8% of the positive samples respectively. In general, salmonellae were isolated from fewer samples of goat samples, probably because of a lower infection rate in the goat population than in sheep. Of the six specimens taken from each animal during the study period, the mesenteric lymph nodes and faecal samples proved to be the most useful indicators of infection. This is because most of the sheep and all of the goats could have been detected on the basis of those samples alone. The liver, spleen, abdominal and diaphragmatic samples did not appear to harbor *Salmonella* in most occasions. This indicate that the organisms did not spread beyond the lymph nodes or if did so were in numbers too small to be detected by the method used (Samuel *et al*., 1980a; Nabbut and Al-Nakhli, 1982). Small numbers of organisms which spread systematically would probably localize in the liver and spleen. It is clear, however, that in healthy animals significant spread beyond the mesenteric lymph nodes does not occur (Samuel *et al*., 1980a).

Salmonella isolation rate of 4.8% in faecal samples of sheep in this study support earlier observations of 4.2% by Kotova *et al.* (1988), 4.0% in India (Kaura and Sharma, 1978) and 3.2% in Egypt (Abdel-Ghani *et al*., 1987). However, it was relatively higher than the 2.2% isolation rate in India (Kapur *et al*., 1982), 2.1% in Ethiopia (Woldemariam, 2003) and 2% in Argentina (Terzolo *et al*., 1979). The 2% prevalence in goat faeces in this study was comparable to that of 3.3% in Ethiopia (Woldemariam, 2003), 2.5% in Italy (Cortesi *et al*., 1984) and 2.2% in Egypt (Abdel-Ghani *et al*., 1987).The higher isolation rate in faeces of sheep in this study clearly indicate the danger of the existence of meat contamination during the slaughtering process in the abattoirs. Usually, healthy carriers intermittently excrete only a few *Salmonella*, unless they undergo some kind of stress (example during transport or holding in the lairages prior to slaughter). It is well established that in stressed group of animals, more animals shed salmonellae and more salmonellae are shed per animal. As a result, there may be an increase in the surface contamination of live animals, contamination of carcasses on the slaughter line, and contamination of the final product (WHO, 1988).

In this study, 7.7% of the sheep examined were harbouring salmonellae in their mesenteric lymph nodes. This high level *Salmonella* isolation in mesenteric lymph nodes may reflect the fact that most of the sheep were held for atleast 4 days before slaughter and they may have originated from a population with a high prevalence of *Salmonella* carrier sheep. Four sheep were detected as positive by faecal (n=2) and abdominal muscle (n=2) samples which were considered negative by mesenteric lymph nodes samples. The mesenteric lymph nodes in the faecal positive animals may have been negative because the animals were passive carriers of organisms that had not invaded the mucosal barriers and reached the lymphatics (Gay *et al*., 1994) whereas in abdominal muscle positive animals, the sheep may have been negative but positivity might come due to contamination during slaughtering process.

The 7.7% detection rate in mesenteric lymph nodes of sheep support the 8.1% finding of Pateraki *et al*. (1975) in Greece and 6.7% in India (Manickam and Victor, 1975). However, the carrier rate of goats in mesenteric lymph nodes was low (only 2% compared with 7.7% in sheep). This is also much lower than 11.6% reported by Woldemariam (2003) in Ethiopia. This might be due to the lower *Salmonella* carrier rate in the present study population and it is likely due to that the goats were held at collection site and/or abattoir holding yards for a shorter period than those slaughtered earlier in Elfora Debre Zeit abattoir, where the infection rate was high. It is relatively lower than the 3.6% found in Senegal (Doutre and Boche, 1976) but comparable to 1.2% carrier rate found in mesenteric lymph nodes of goats in Sri Lanka (Subasinghe and Ramakrishnaswamy, 1983). On the ground that 66.7% of the infected sheep and 66.7% of the infected goats had salmonellae in their mesenteric lymph nodes (Table 6 and 7) it was likely that most of the infected sheep and goats harbored their infections while still on the farms of origin. Carrier sheep and goats could serve as sources of *Salmonella* contamination during and after transportation to a slaughterhouse increasing the risk that the meat will be contaminated after slaughter. Because carrier sheep and goats do not have any clinical abnormalities, identification of these animals is difficult and requires laborious bacteriologic culture techniques.

In carrier animals the lymph nodes may harbor large numbers of salmonellae (Moo *et al*., 1980; Samuel *et al*., 1980a). It has been suggested that meat inspector's knives may also contribute to the *Salmonella* contamination of the carcasses (Smeltzer and Thomas, 1981). When lymph nodes from infected animals are incised during the normal procedure of meat inspection, a substantial reservoir of salmonellae would be exposed and transferred to other parts of the carcasses via infected implements or personnel could easily ensue (Moo *et al*.,

1980). The finding of high proportion of infected sheep and goats harboring salmonellae in their mesenteric lymph nodes in this study indicate the existence of a substantial risk of cross-contamination possibly during slaughtering, subsequent handling, storage and distribution of the carcasses. Abattoir personnel and meat inspectors need special instruction on the necessity of hygienic operation during slaughtering and incision for inspection in order not to contaminate the carcasses through knives and hands.

The low isolation rates in liver and spleen of sheep in this study support the findings of Kumar *et al*. (1973), Nabbut and Al-Nakhli (1982) and Subasinghe and Ramakrishnaswamy (1983). The low detection rates in these organs indicate that localization of the organisms in these organs is most likely minimal. It appeared to be rare for the liver and spleen tissue to be infected with *Salmonella* before death. The liver and spleen is usually free of *Salmonella* at slaughter but the surface becomes contaminated at some time there after. The ultimate source of this contamination is likely to be the *Salmonella* present in the gastrointestinal tract and mesenteric lymph nodes either of the same animal or of other animals slaughtered on the same day (Samuel *et al*., 1980b; Nabbut and Al-Nakhli, 1982).

It is generally accepted that the internal tissues of healthy slaughter animals are free of bacteria at the time of slaughter, assuming that the animals are not in a state of exhaustion (Jay, 2000). However, due to many reasons, microorganisms are recovered in the internal tissue of slaughtered animals. In the present study, salmonellae were isolated in 1.9% of the abdominal muscle of sheep while it was not detected in any of the diaphragmatic muscle of sheep and both diaphragmatic and abdominal muscles of goats. This indicate low contamination rate during slaughtering. This finding is much lower than the 13.1% from sheep carcasses in meat processing plants in Russia (Kotova *et al*., 1988), 10% from freshly dressed carcasses in Spain (Sierra *et al*., 1995) and 12% from slaughtered goats in India (Arora, 1978; Das *et al*., 1990) and lower than the findings of Moo and colleagues (1980), who reported an average *Salmonella* infection rate of 5% in mutton, isolation of 5.56% in mutton (Poernomo, *et al*., 1986), carcass contamination of 7.4% in sheep and 7.5% in goats (Woldemariam, 2003). These findings indicate that there was severe contamination during slaughter. The probable source could have been from poorly disinfected knives or other slaughtering equipment and poor hygiene of the slaughterhouse personnel on cross-contamination (Smeltzer and Thomas, 1981; Smeltzer *et al*., 1980).

5.2. Distribution of *Salmonella* Serotypes

Out of the total 22 *Salmonella* isolates, 9 different *Salmonella* serotypes were identified in sheep and goats (Table 10). Some of the serotypes isolated in this study (*S*. Typhimurium, *S*. Poona, *S*. Heidelberg, *S*. Reading and *S*. Enteritids) were also reported by different workers in sheep and goats of different countries (Kumar *et al*., 1973; Nabbut and Al-Nakhli, 1982; Kotova *et al*., 1988; Adesiyun *et al*., 1988; Woldemariam, 2003). Previously other workers have reported some of the isolates in animals and their products in Ethiopia (Pegram *et al*., 1981; Molla et al., 2003a). However, the three isolates, *S*. Poona, *S*. I: 6, 7, 14:-:enz15 and *S*. Niederoderwitz had not been previously reported in Ethiopia.

Salmonella Typhimurium was the most prevalent serotypes in this study. This finding is consistent with that of Sierra *et al*. (1995) who found *S*. Typhimurium predominantly in fresh dressed lamb carcass. McOrist and Miller (1981) reported *S*. Typhimurium as a significant cause of mortality in large number of feral goats following their prolonged transport and intensive handling, Kane (1979) isolated mainly *S*. Typhimurium from apparently healthy slaughtered sheep. Kumar *et al*. (1973) and Nabbut and Al-Nakhli (1982) also reported high isolation rate of *S*. Typhimurium in sheep and goats tissue and faecal samples. *Salmonella* Typhimurium is a common serovar also isolated from all species of animals and birds, and has been shown to cause severe losses. In Ethiopia, *S*. Typhimurium was reported from edible chicken offals (Molla *et al*., 1999a), in bovine mesenteric lymph nodes and beef cuts (Alemayehu *et al*., 2003). The isolation of *S*. Typhimurium in the present study confirms continued existence of this serotype in the livestock population of Ethiopia.

Though *S*. Poona was isolated for the first time in Ethiopia, it has been commonly found in sheep and goats of other countries. In Saudi Arabia, Nabbut and Al-Nakhli (1982) isolated *S*. Poona in apparently healthy slaughtered sheep and goats at Riyadh abattoir. In India, Kumar *et al*. (1973) reported in apparently healthy slaughtered goats and in Nigeria, Falade (1976) isolated *S*. Poona from faeces of healthy goats slaughtered at Kanu abattoir. Adesiyun *et al*. (1988) also isolated *S*. Poona from slaughtered goats in Zaria, Nigeria.

Eventhough the data were insufficient to indicate whether or not any serotype was predominantly associated with one or other animal species or abattoir, the range of serotypes isolated in this study vary considerably between species and abattoirs in their presence and frequency of detection (Table 8). Of the 9 identified serotypes, *S*. Typhimurium var.

Copenhagen, *S*. Heidelberg, *S*. Reading, *S*. Give, *S*. Enteritids, *S*. Niederoderwitz were detected only in sheep. *Salmonella* Poona and *S*. I: 6, 7, 14:-:enz15 were isolated only in goats, while *S*. Typhimurium was recovered in both species. The basis of these differences remains to be defined. One possible cause may be the animal feed. Raw materials for animal feed are generally thought to be an important source of salmonellae. Other possible reasons for the observed difference in distributions of *Salmonella* serotypes among sheep and goats are differences in housing, husbandry and management practices in sheep and goat production. Furthermore, even if the difference in *Salmonella* prevalence between the two abattoirs was not statistically significant, the serotypes identified in Addis Ababa and Modjo abattoir vary extensively (Table 8).

Most of the serotypes isolated in this study are incriminated to cause major foodborne outbreaks of human salmonellosis in different countries of the world (D'Aoust, 1997; Jay, 2000). The isolation of serovars belonging to the somatic serogroups B, C and E reiterates their importance in the global food chain, and recalls their prominent association with human salmonellosis (D'Aoust, 1989; D'Aoust *et al*., 1992). *Salmonella* Typhimurium, *S*. Heidelberg and *S*. Enteritidis are listed within the five most prevalent *Salmonella* serovars reported from different sources (Jay, 2000). Furthermore, they are the first three in the 11 top ranked salmonellae serovars isolated from clinical specimens in the United States for 1996-1997 (CDC, 1998).

Changes in the relative frequency of serotypes can be observed over short periods of time, sometimes within one or two years (Acha and Szyfres, 2001). Eleven different *Salmonella* serotypes have been previously identified from sheep and goats (Woldemariam, 2003) and mutton (Ejeta *et al*., 2004). However, from those serotypes identified previously, it is only *S*. Tphimuriumin recovered again in the present study while others were not among the nine serotypes included in the present finding. It is possible that those serotypes could be present in Ethiopian sheep and goats in such low magnitude that our present sample size was not large enough to detect them or they might be restricted in limited geographical areas and small ruminant population of the country. It is known that the presence and distribution of *Salmonella* serotypes could vary geographically from region to region. While some serovars maintain their dominance role over many years, others emerge or decreased over time (ICMSF, 1996). The rapid international trade in agricultural, acquacultural and food products has also facilitated the introduction of new *Salmonella* serotypes into importing countries (D'Aoust, 1994).

5.3. Antimicrobial Resistance Pattern of *Salmonella* Isolates

Antimicrobial resistant *Salmonella* isolates from animals and human sources have been reported in Ethiopia (Molla *et al*., 1999b; Tibaijukan *et al*., 2002; Mache, 2002; Alemayehu *et al*., 2003; Molla *et al*., 2003b). In the present study 31.8% of the *Salmonella* isolates from sheep and goats were multiple resistant to antimicrobial drugs commonly used to treat bacterial infections in domestic animals and human beings in Ethiopia. This finding supports the well known fact that antimicrobial resistance rate of *Salmonella* isolates in Sub-Saharan Africa is generally high which is primarily attributed to the indiscriminate use of antimicrobials both in animals and human health sectors (Leegaard *et al*., 1996; Molla *et al*., 1999b).

In the present study, *Salmonella* isolates showed substantial resistance to ampicillin, cephalothin, streptomycin, sulfisoxazole, tetracycline, sulfamethoxazole/trimethoprim, trimethoprim, amoxicillin/clavulanic acid, chloramphenicol and spectinomycin. The resistance to these antimicrobials was considerably high and varied from 4.6% to 18%. These findings were not totally unexpected given the indiscriminate use of antimicrobials in animal husbandry. This result supports the findings of Molla *et al*. (1999b) and Alemayehu *et al*. (2003), who reported antimicrobial resistant *Salmonella* isolates from chicken and minced beef, and apparently healthy slaughtered cattle respectively, resistant to the above mentioned antimicrobials. Mache *et al*. (1997) and Mache (2002), detected *Salmonella* isolates resistant to tetracycline, ampicillin, cephalothin, trimethoprime-sulfamethoxazole, chloramphenicol and kanamycin from human sources.

All the resistant strains (62.5%) of *S*. Typhimurium were exhibited multiple drug resistance to ampicillin, cephalothin, streptomycin, sulfisoxazole, trimethoprim, tetracycline, sulfamethoxazole/trimethoprim, amoxicillin/clavulanic acid, chloramphenicol and spectinomycin (Table 13). Gebre-Yohannes *et al*. (1987) reported multiple drug resistant *S*. Typhimurium strains to ampicillin, chloramphenicol, cephalothin, kanamycin, neomycin, streptomycin, sulphadiazin, tetracycline and trimethoprim from hospitalized patients in Addis Ababa. In this study both the two isolates of *S*. Reading (100%) were resistant to streptomycin, sulfisoxazol and tetracycline. This supports the finding of Corrier (1989), who reported *S*. Reading as the most resistant serotype from feeder calves with all isolates being resistant to 10 or 11 of 13 antimicrobial drugs tested.

Multiple drug resistant *Salmonella* isolates have been reported since the 1960, with the resistance patterns of *Salmonella* serovars of public health importance often associated with specific phage types. *Salmonella* Typhimurium phage type 193 is a notable multiple drug resistance strain responsible for outbreaks in humans in the late 1980s and early 1990s, mainly in Europe (Gebreyesus and Altier, 2002). This phage type has been among the most common multiple drug resistant strains that have been identified from sheep and goats in this study. This phage type exhibited a multi-drug resistance to up to 9 antimicrobials (AmpAmcCefChlSptStrSulSxtTmp). It has already been intimated that emergence of multiply-resistant salmonellae can be the product of conjugative transfer of R-plasmids between bacterial species. Agricultural use of subtherapeutic doses of a single antibiotic could select for bacterial strains harboring plasmids with multiple resistance codons (D'Aoust, 1991; D'Aoust, 1997). The emergence and prevalence of multiply-resistant salmonellae in meat animals can seriously compromise public health (D'Aoust *et al*., 1992).

5.4. Synopsis

The detection of invasive, multiple drug resistant and pathogenic *Salmonella* serotypes such as *S*. Typhimurium, *S*. Heidelberg, *S*. Enteritidis, S. Reading and others in this study indicate the public health significance of these serovars as contaminated sheep and goat meat and meat products may pose health hazards. The high risk part of human population that is infants, elderly, immunocompromized such as individuals with HIV/AIDS and malnourished persons are highly susceptible and the presence of *Salmonella* even in low numbers constitute a major public health concern (D'Aoust, 1989; Doyle and Cliver, 1990; Bryan and Doyle, 1995). This risk may further be accentuated if sheep and goats meat is consumed raw or under cooked.

The data obtained in this study and those of Woldemariam (2003) and Ejeta *et al*. (2004) indicated that a wide range of *Salmonella* serotypes occur in sheep and goats population of Ethiopia. This underlines the significance of *Salmonella* for livestock producers as well as for consumers. In this country sheep and goats live in close proximity or even together with humans and could contaminate drinking water and the environment with salmonellae. In the community, backyard slaughter is a common practice without antemortem and postmortem inspections. The slaughtering of such carrier animals under unhygienic conditions (lack of water, unclean water supply, dirty knives and hands, lack of chilling facilities, etc.) may lead to gross contamination of the carcasses with salmonellae, thus increasing the risk to the

human population. Furthermore, the problem is more complicated by the raw meat consumption habit of the community. A considerable proportion of the Ethiopian populations have the habit of consuming raw meat. Improper disposal of human excreta, the high temperature and humidity, the practice of keeping carcasses together with offals and the presence of high density flies further facilitate the spread of the infection in the population in the country.

6. CONCLUSIONS AND RECOMMENDATIONS

The investigation of *Salmonella* in sheep and goats at Addis Ababa public and Modjo modern export abattoirs showed a high (11.5%) and relatively low (3%) prevalence of *Salmonella* infection in apparently healthy slaughtered sheep and goats respectively. Higher isolation was observed in mesenteric lymph nodes and faeces of sheep and goats samples as compared to other sample types. *Salmonella* carrier was not detected from apparently healthy abattoir personnel of both Addis Ababa public and Modjo Modern export abattoirs.

A total of 22 *Salmonella* isolates, consisting of 9 different serotypes were identified from sheep and goats. The most prevalent serotypes isolated were *S*. Typhimurium, followed by *S*. Heidelberg, *S*. Reading, *S*. Give, and *S*. Poona. Three serotypes, S. Poona, S. I: 6, 7, 14:-:enz15 and S. Niededrowtiz had not been previously reported in Ethiopia. Five of the serotypes, which had not been isolated previously from sheep and goats in Ethiopia, were identified.

A total of 7 isolates (31.8%) were resistant to two or more antimicrobial drugs. Of the *Salmonella* serovars isolated, only strains of *S*. Typhimurium and *S*. Reading were resistant. Both strains showed a multiple drug resistance pattern to up to 9 different antimicrobials. Of the *S*. Typhimurium strains, phage type 46 and 193 were resistant. Resistance was observed to ampicillin, cephalothin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline, sulfamethoxazol/trimethoprim, trimethoprim, amoxicillin/clavulanic acid and spectinomycin.

The existence of *Salmonella* in sheep and goats in general and the isolation of multi-drug resistant and pathogenic *Salmonella* serotypes such as *S*. Typhimurium, *S*. Heidelberg, *S*. Enteritidis and S. Reading in particular emphasizes the threat to public health. This is higher particularly to those who have direct or indirect contact to sheep and goats (farmers, abattoir workers, animal health personnel) and to consumers who have the habit of eating raw or under cooked meat.

Salmonellosis is a zoonotic disease in which foods of animal origin is an important source of human infection. In many countries, there has been a significant increase in human salmonellosis in recent years. The disease constitutes an important problem, even in those countries with high standards of hygiene. The export trade in meat and animal products in Ethiopia will depend on continued compliance with stringent standards of hygiene expected

by importing countries. To control and prevent *Salmonella* infection and contamination in live animals and animal products, it is critical that risk reduction strategies should be used throughout the food chain that is from farm to fork. Based on the findings of the present study, the followings are recommended:

- Ligation of the esophagus and gut during evisceration, and immediate separation of the offal from the carcasses should be employed. .
- Standard abattoir and personal hygiene should be employed to control contamination of meat and meat products with *Salmonella*.
- Awareness should be created among the public about the risks associated with a consumption of raw or undercooked meat.
- Antimicrobial agents must be used wisely both in animals and humans.

7. REFERENCES

Abdel-Ghani, M., Mohomed, A. H., Yassein, S. (1987): Occurrence of *Salmonella* in sheep and goats in Egypt. *J. Egyp. Vet. Med. Assoc*. **47**, 161-170.

Acha, P. N. and Szyfres, B. (2001): Zoonoses and Communicable Diseases Common to Man and Animals. 3rd ed. Vol. I. Bacteriosis and Mycosis. Washington DC: Pan American Health Organization. PP. 233-246.

Adesiyun, A. A., Dasuki, M. O., Ibrahim, G. A. (1988): Occurrence and antibiograms of salmonellae isolated from slaughter sheep and goats in Zaria, Nigeria. *Israel J. Vet. Med.* **44**, 248-255.

Aiello, S. E. and Mays, A. (1998): The Merck Veterinary Manual. 8th ed. U.S.A.: Merck and Co. Inc. White Houses Station. PP. 120-123.

Alemayehu, D., Molla, B. and Muckle, A. (2003): Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle in Ethiopia. *Trop. Anim. Hlth. Prod*. **35**, 309-319.

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

Anderson, E. S. (1964): The phage typing of *Salmonella* other than *S*. Typhi. In: Van Oye E. (ed): The World Problem of Salmonellosis. The Hague, The Netherlands: Dr. W. Junk Publishers. PP. 89-100.

Anderson, E. S., Ward, L. R., De Saxe M. J., De Sa J. D.H. (1977): Bacteriophage-typing designations of *Salmonella* Typhimurium. *J. Hyg*. **78,** 297-300.

Andreani, E., Prosperi, S., Arush, M. A., Salim, A. H. (1979): *Salmonella* carriers among cattle, sheep, goats and dromedaries in the Somalia Democratic Republic. *Annali della Facotta di Medicina Veterinaria di Pisa,* **31**, 65-72.

Arora, A. K. (1978): Recovery of *Salmonella* serotypes from various sources in slaughtered goats. *Indian J. Public Health,* **22**, 305-306.

Asfaw, W. (1998): Country report (Ethiopia). In: CTA (ed.): Livestock development policies in Eastern and Southern Africa. Proceedings of a seminar organized by CTA, OAU/IBAR and the Ministry of Agriculture and Cooperatives, Swaziland, held at Mbabone, Swaziland, 28 July-1 August 1997. UK: Ebenezer Baylis, the Trinity Press. PP. 216-220.

Ashenafi, M. and Gedebou, M. (1985): *Salmonella* and *Shigella* in adult diarrhea in Addis Ababa: prevalence and antibiograms. *Trans. Royal Society Trop. Med.Hyg.* **79**, 719-721.

Baird-parker, A. C. (1990): Foodborn salmonellosis. *The Lancet,* **336**, 1231-1235.

Bean, N. H., Griffin, P. M., Goulding, J. S. Ivey, C. B. (1990): Foodborne disease outbreaks, 5-years summary, 1983-1987. *J. Food Prot.* **53**, 711-728.

Bhatia, R. and Ichhpujani, R. L. (1994): Essential of Medical Microbiology. New Delhi: Jaypee Brothers (p) Ltd. PP. 367-380.

Blackburn, C. de. W. (1993): Rapid and alternative methods for the detection of salmonellas in foods. *J. Appl. Bacteriol*. **75**, 199-214.

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

Bryan, F. L. and Doyle, M. P. (1995): Health risk and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *J. Food Prot*. **58**, 326-344.

Callow, B. R. (1959): A new phage typing scheme for *Salmonella* Typhimurium. *J. Hyg*. **57**, 346-359.

Centers for Disease Control and Prevention (CDC) (1998): Multistate outbreak of *Salmonella* serotypes Agona infections limited to toasted oats cereal-United States. *Morb. Mort. Wkly. Rep*. **47,** 462-464.

Central Agricultural Census Commission (CACC) (2003): Statistiacal report on livestock and farm implements. Ethiopian Agricultural Sample Enumeration, 2001/02. Addis Ababa, Ethiopia.

Clarke, R. C. and Gyles, C. L. (1993): *Salmonella*. In: Gyles, C. L. and Thoen, C. O. (eds.): Pathogenesis of Bacterial Infections in Animals. 2nd ed. USA: Iowa State University press, Ames. PP. 133-153.

Corrier, D. E. (1989): Effects of marketing stress on fecal excretion of *Salmonella* species in feeder calves. *Am. J. Vet. Res*. **51**, 866-869.

Cortesi, M. L., Brillante, M., Giovanni, F. DE. (1984): *Salmonella* carriage by apparently healthy slaughter sheep and goats. *Archivio Vetrinario Italiano*. **35**, 258-261.

Cortinez, Y. J. M. DE. and Guzman, A. M. S. DE. (1987): Investigation of *Salmonella* in slaughtered kids. *Boletin de la Oficina Sanitaria Panamericana*, **103**, 373-377.

D'Aoust, J.-Y. (1989): *Salmonella*. In: Doyle, M. P. (ed.): Foodborne Bacterial Pathogens. New York: Marcel Dekker. PP. 328-413.

D'Aoust, J.-Y. (1991): Pathogenicity of foodborne *Salmonella. Int. J. Food Microbiol*. **12**, 17-40.

D'Aoust, J.-Y., Sewell, A. M., Dally, E. and Greco, P. (1992): Antibiotic resistance of agricultural and foodborne *Salmonella* isolates in Canada: 1986-1989. *J. Food Prot.* **55**, 428-434.

D'Aoust, J.-Y. (1994): *Salmonella* and the international food trade. *Int. J. Food Microbiol.* **24**, 11-31.

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

Das, M. S., Roy, D. K., Dash, S. (1990): Occurrence of salmonellae in slaughtered pigs, goat meat, meat handlers and slaughtered house workers. *J. Communi. Dis.* **22**, 39-42.

Doutre, M. P. and Boche, R. (1976): Serotypes de *Salmonella* isoles chez les petits ruminants abattus a Dakar. *Revue d'Elevage et de medicine veterinaire des pays Tropcaux (France*). **29**, 205-209.

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

Ejeta, G., Molla, B., Alemayehu, D., Muckle, A. (2004): *Salmonella* serotypes isolated from minced beef, mutton and pork in Addis Ababa, Ethiopia. *Rev. Med. Vet*. (In press).

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.

Falade, S. (1976): *Salmonella* serotypes from slaughterhouse at Kano abattoir: A short communication. *Indian J. Public Health*. **24**, 265-266.

Gay, J. M., Rice, D. H., Stieger, J. H. (1994): Prevalence of faecal *Salmonella* shedding by cull dairy cattle marketed in Washington State. *J. Food Prot*. **57**, 195-197.

Gebreyes, W. A. and Altier, C. (2002): Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolates from swine. *J. Clin. Microbiol.* **40**, 2813-2822.

Gebre-Yohannes, A., Mamo, K., Wolde, H. (1987): R-factor mediated multi-drug resistance in *Salmonella* Typhimurium isolates. *Ethiop. Med. J*. **25,** 53-54.

Hemmingsen, P., Montgomery, R. A., Ward, C. G., Henderson, T. G. (1982): A fatal disease outbreak in sheep associated with *Salmonella* enteritids ser. Havana. *N. Z.Vet. J.* **30**, 98.

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

International Commission on Microbiological Specification for Foods (ICMSF) (1996): Salmonellae. In: ICMSF (ed.). Microorganisms in Foods. London: Chapman and Hall. PP. 126-140.

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

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

Jawetz, E., Adelberg's, E. A., Mlnick, J. L. (1995): Medical Microbiology. 20th ed. Appleton and Large. PP. 206-217.

Kane, D. W. (1979): The prevalence of *Salmonella* infection in sheep at slaughter. *N. Z. Vet. J.* **27**, 110-113.

Kapur, M. P., Kulshreshtha, R. C., Kalra, D. S. (1982): *Salmonella* serotypes from sheep and their significance. *Indian Vet. J.* **59**, 331-334.

Kariuki, S., Cheesbrough, J., Mavridis, A. K., Hart, C. A. (1999): Typing of *Salmonella enterica* serotype *paratyphi* C isolates from various countries by plasmid profiles and pulsed-field gel electrophoresis. *J. Clin. Microbiol*. **37**, 2058-2060.

Kaura, Y. K. and Sharma, V. K. (1978): Isolation of salmonellae from lambs. *Indian J. Experimental Biology,* **17**, 448-450.

Khakhria, R., Woodward, D., Johnson, W. M., Poppe, C. (1997): *Salmonella* isolated from humans, animals and other sources in Canada during the period 1983-1992. *Epidemiol. Infect.* **119**, 15-23.

Kimberling, C. V. (1988): Jensen and Swift's Diseases of Sheep. 3rd ed. Philadelphia: Lea and Febiger. PP. 54-55, 153-154.

Kotova, A. L., Kondratskaya, S. A., Yasutis, I. M. (1988): *Salmonella* carrier state and biological characteristics of the infectious agent. *J. Hyg., Epid., Micro. Immu.* **32**, 71-78.

Kumar, S., Saxena, S. P., Gupta, B. K. (1973): Carrier rate of salmonellas in sheep and goats and its public health significance. *J. Hyg. Comb.* **71**, 43-47.

Leegaard, T. M., Van Gestel, M. H., Petit, P. L. C., Van De Klundert, J. A. M. (1996): Antibiotic resistance mechanisms in *Salmonella* species causing bacteraemia in Malawi and Kenya. *APMIS*, **104**, 104-306.

Leondidis, S., Christopoulos, C., Burtzi-Htzopoulou, Sarris, K., Xenos, G. (1984): Abortion and losses of newborn lambs and kids due to *Salmonella* species [Greece]. In priority aspects of salmonellosis research. A workshop held in Brussels, 11-13 October 1983 (edited by H. E. Larsen). Luxembourg: Commission of European Communities, PP. 183-186.

Linklater, K. A. (1983): Abortion in sheep assosciated with *Salmonella* Montevideo infection. *Vet. Rec.* **112**, 372-374.

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

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. *Ethiop. J. Health Sci.* **12**, 37-46.

Manickam, R. and Victor, D. A. (1975): A study on the occurrence of *Salmonella* in abattoir carcasses. *Indian Vet. J.* **52**, 44-47.

Mcauliffe, P. R., Hardefeldt, K. W., Hucker, D. A. (1978): Health problems associated with the export of live sheep. *Aust. Vet. J.* **54,** 594-595.

McOrist, S. and Miller, G. T. (1981): Salmonellosis in transported feral goats. *Aust. Vet. J.* **57**, 389-390.

Mered, B., Boulmerka, Z., Benelmouffok, A., Dedet, J.-P., Semeri, R., Benyahia, Y. (1977): Survey of *Salmonella* in animals in Algeria. *Archives de l'Institute Pasteur d'Algerie,* **52**, 5-16.

Molla, B., Kleer, J., Sinell, H. J. (1999a): Occurrence, distribution and level of *Salmonella* in selected food items in Addis Ababa (Ethiopia). *Fleischwirtschaft. International,* 37-39.

Molla, B., Kleer,J., Sinell, H. J. (1999b): Antibiotics resistance pattern of foodborne *Salmonella* isolates in Addis Ababa, (Ethiopia). *Berl. Munch. Tierärztl. Wschr.* **112**, 41-43.

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

Molla, B., Mesfin, A., Alemayehu, D. (2003b): Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. *Ethiop. J. Health Dev*. **17**, 131-139.

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 Universitat Berlin and Addis Ababa University, MSc. Thesis.

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

Nabbut, N. H. and Al-Nakhli, H. M. (1982): Incidence of salmonellae in lymph nodes, spleen and faeces of sheep and goats slaughtered in the Riyadh public abattoir. *J. Food Prot.* **45**, 1314-1317.

Nabbut, N. H., Barbour, E. K., Al-Nakhli, H. M. (1982): *Salmonella* species and serotypes isolated from farm animals, animal feed, sewage, and sludge in Saudi Arabia. *Bull. World Health Organ.* **60**, 803-807.

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.

National Meteorological Centre Agency (NMSA) (1999): Rain fall, humidity and temperature data. Addis Ababa, Ethiopia.

Nyeleti, C., Hildebrandt, G., Kleer, J., Molla, B., (2000): Prevalence of *Salmonella* in Ethiopian cattle and minced beef. *Berl. Munch.Tierärztl.Wschr.* **113**, 431-434.

Office International des Épizooties (OIÉ) (2000): Salmonellosis. In: Manual of Standards for Diagnostic Testes and Vaccines, OIÉ. Paris. PP. 832-842.

Old, D. C. (1990): *Salmonella*. In: Parker, M. T. and Duerden, B. I. (eds.): Systematic Bacteriology. 8th ed. vol. 2. London: Edward Arnold. PP. 469-489.

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

Pateraki, E., Auramidis, D., Trichopoulou, A., Papaiconomou, N., Georgiu, E., Vassiliads, P. (1975): Salmonellas in the mesenteric lymph nodes of pigs, calves and sheep slaughtered at Athens abattoir. *Archives de l'institut Pasteur Hellenique.* **21**, 31-45.

Pegram, R. G., Roeder, P. L., Hall, M. L. M., Rowe, B. (1981): *Salmonella* in livestock and animal byproducts in Ethiopia. *Trop. Anim. Hlth. Prod.* **13**, 203-207.

Person, U. and Jendteg, S. (1992): The economic impact of poultry borne salmonellosis: how much should be spent on prophylaxis? *Int. J. Food Microbiol*. **15**, 207-213.

Poernomo, S., Hardjoutomo, S., Istiana, Hirst, R. G. (1986): *Salmonella* isolation from slaughtered sheep and goats and the abattoir environment in Bogor and Jakarta. *Penyakit Hewan*, **18**, 24-29.

Popoff, M, Y., and Le Minor, L. (1997): Antigenic Formulas of the *Salmonella* Serovars. 7th ed. WHO Collaborating Centre for Research on *Salmonella.* Institute Pasture. Paris, France.

Quinn, P. J., Carter, M. E., Markey, B. K., Carter, C. R. (1994): Veterinary Clinical Microbiology. London: Wolfe Publishing. PP. 226-234.

Radostits, O. M., Blood, D. C., Gay, C. C. (1994): Veterinary Medicine: A Text Book of the Disease of Cattle, Sheep, Pigs, Goats and Horse. 8th ed. London: Bailliere Tindall. PP. 730-747.

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

Samuel, J-L., O'Boyle, D. A., Mathers, W. J., Frost, A. J. (1980b): The contamination with *Salmonella* of bovine livers in an abattoir. *Aust. Vet. J.* **56**, 526-528.

Samuel, J-L., Eccles, J. A., Francis, J. (1981): *Salmonella* in the intestinal tract and associated lymph nodes of sheep and cattle. *J. Hyg*. **87**, 225-232.

Seifert, H. S. H. (1996): Tropical Animal Health. Dorotrecht: Kluwer Academic Publishers. PP. 368-371.

Sewell, M. M. H. and Brocklesby (1990): Handbook on Animal Diseases in the Tropics. 4th ed. Lndon: Baillier Tindall. PP. 92-96.

Sharma, R. N., Musonda, M. M., Munang'andu, H. M., Muyoyeta, P., Sinyangwe, P. G. (1996): *Salmonella* isolation from animals in the Republic of Zambia. *Bull. Anim. Hlth. Prod. Afri*. **44**, 5-7.

Shipp, C. R. and Rowe, B. (1980): A mechanised microtechnique for *Salmonella* serotyping. *J. Clin. Pathol.* **33,** 595-597.

Sierra, M.-L., Gonzalez-Fandos, E., Garcia-Lopez, M.-L., Fernandez, M. C. G., Prieto, M. (1995): Prevalence of *Salmonella, Yersinia, Aeromonas, Campylobacter,* and cold-growing *Escherchia coli* on freshly dressed lamb carcasses. *J. Food Prot*. **58**, 1183-1185.

Singh, B. P. and Arora, A. K. (1981): Prevalence of *Salmonella* serotypes in goat-meat. *Acta Veterinaria Yugoslavia, **31***, 243-250.

Smeltzer, T., Thomas, R., Collins, G. (1980): The role of equipment having accidental or indirect contact with the carcase in the spread of *Salmonella* in an abattoir. *Aust. Vet. J*. **56**, 14-17.

Smeltzer, T. and Thomas, R. (1981): Transfer of salmonellae to meat and offal by knives. *Aust. Vet. J.* **57**, 433-434.

Smith, M. C. and Sherman, D. M. (1994): Goat Medicine. Maryland: Williams and Wilkins. PP. 302-305.

Sockett, P. N. (1991): The economic implication of human *Salmonella* infection. *J. Appl. Bacteriol.* **71**, 289-295.

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

Stohr, K. (1995): Cost/benefit analysis of control measures directed at prevention of Salmonellosis. MZCPC Consultation on Human and Animal Salmonellosis. National Control Activities in the MZCP countries. Mediterranean Zoonoses Control Programme. WHO. 51p. (MZCP/SALM 95.1).

Subasinghe, D. H. A. and Ramakrishnaswamy, A. (1983): Salmonellosis in Sri Lanka: Isolation of salmonellae from goats slaughtered at an abattoir. *Sri Lanka Vet. J.* **31**, 40-42.

Tadjbakhch, H., Baba-Ali, G. H., Haghigat, M. (1992): Prevalence of *Salmonella* carriers among sheep and dromedaries in Iran. *Rev. Medi. Vét.* **143**, 361-365.

Tegegne, M. and Ashenafi, M. (1998): Microbial load and incidence of *Salmonella* Spp. in 'kitfo', a traditional Ethiopian spiced, minced meat dish. *Ethiop. J. Health Dev.***12**, 135-140.

Terzolo, H. R., D'Empaire, M., Bendersky, S., Igarza, L. M. (1979): Prevalence of *Salmonella* Typhimurium in clinically healthy flock of sheep. *Revista de Medicina Veterinaria, Argentina,* **60**, 277-282.

Thrusfield, M. (1995). Veterinary Epidemiology. 2nd ed. Oxford: Black Well Science Ltd. PP. 182-198.

Tibaijuka, B., Molla, B., Hildebrand, G., Kleer, J., Salah, W. (2002): Antimicrobial resistance to salmonellae isolated from retail raw chicken meat and giblets in Ethiopia. *Bull. Anim. Hlth. Prod. Afr.* **50**, 86-95.

Tibaijuka, B., Molla, B., Hildebrandt, G., Kleer, J. (2003): Occurrence of salmonellae in retail raw chicken products in Ethiopia. *Berl. Munch. Tierärztl. Wschr.* **116**, 55-58.

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

Uzoukwu, M. and Mohan, K. (1990): Intestinal pathology Associated with *Salmonella* in apparently healthy animals. Bull. *Anim. Hlth. Prod. Afri.* **38,** 351-353.

Varnam, A. H. and Evans, M. G. (1991): Foodborne Pathogens: An Illustrated Text. England: Wolfe Publishers Ltd. PP. 51-85.

Verma, S., Katoch, R. C., Verma, J. C., Nigam, P., Batta, M. K. (1998): Reproductive failures in does and ewes due to salmonellosis in Himachal Pradesh. *India Vet. Arhiv*. **68**, 177-181.

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

Wernery, U. and Thimm, B. (1978): Isolation of 54 salmonellae strains including 14 serotypes from domestic animals in the Somali Democratic Republic. *Zentralblatt für Veterinamedizin,* **25B**, 539-546.

Wierup, M. (1995): Control of *Salmonella* infections in animals-the primary source of salmonellosis in humans. In: Lindberg, R. (ed.) Veterinary medicine-impacts on human health and nutrition in Africa. Proceeding of an International Conference held at ILRI, Addis Ababa, Ethiopia, 27-31, Aug. 1995. PP. 33-39.

Woldemariam, E. (2003): Prevalence and distribution of *Salmonella* in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. AAU, FVM, Debre Zeit, Ethiopia, DVM. Thesis.

World Health Organization (1988): Salmonellosis Control: Role of animal and product hygiene. Report of a WHO Expert Committee. Geneva: WHO Technical Report Series, No. 774.

Wray, C., McLaren, I. M., Beedell, Y. E. (1993): Bacterial resistance monitoring of salmonellas isolated from animals, national experience of surveillance schemes in the United Kingdom. *Vet. Microbiol.* **35,** 313-319.

Wray, C. and Davis, R. H. (2000): *Salmonella* infection in cattle. In: Wray, C. and Wray, A. (eds.): *Salmonella* in Domestic Animals. CABI Publishing UK. PP. 169-190.

Wray, C. and Davis, R. H. (2003): The epidemiology and ecology of *Salmonella* in meat-producing animals. In: Torrence, M. E. and Isaacson, R. E. (eds): Microbiological Food Safety in Agriculture Current Topics. Iowa State: Black Well Publishing Company. PP. 73-82.

8. APPENDICES

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

1. Buffered peptone water (BPW) (Sifin, Berlin, Germany)

Typical 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 media was dissolved in one liter of distilled water and sterilized by autoclaving at 121°C for 15 minutes.

2. Magnesium chloride malachite green broth acc. to Rappaport-Vassiliadis (RV-Medium) (Sifin, Berlin, Germany)

Typical composition (g/liter): Peptone from casein and soy 5.0; magnesium chloride (anhydrous) 18.73; sodium chloride 8.0 potassium dihydrogen phosphate1.6; malachite green 0.04

Preparation: Thirty three point four (33.4) grams of RV-Medium was dissolved in one liter of distilled water, mixed well and sterilized by autoclaving for 20 minutes at 115 ^{O}C.

3. Selenite cystine enrichment broth (Merck, Darmstadt, Germany)

Typical composition (g/liter): Peptone from casein 5.0; L (-) cystine 0.01; lactose 4.0; sodium phosphate 10.0; sodium hydrogen selenite 4.0

Preparation: Twenty-three grams of selenite cystine was dissolved in one liter of distilled water and heated (max. 60 ^{O}C) until it dissolves completely.

4. Brilliant green-phenol-red agar acc. to Edel and Kampelmacher (BPLS Agar, mod.) (Sifin, Berlin, Germany)

Typical 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. XLD-agar (Xylose lysine desoxycholate agar) (Sifin, Berlin, Germany)

Typical 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. Nutrient agar (Oxoid, Hampshire, England)

Typical composition (g/liter): 'Lab-Lemco' powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0

Preparation: Twenty-eight grams of this media was suspended in one liter of distilled water. Brought to the boil to dissolve completely and sterilized by autoclaving at 121 ^{O}C for 15 minutes and then poured into Petri dishes.

7. Triple sugar iron agar (Merck, Darmstadt, Germany)

Typical composition (g/liter): Peptone from casein 15.0; peptone from meat 5.0; meat extract 3.0; yeast extract 3.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; D (+) glucose 1.0; ammonium iron (iii) citrate 0.5; sodium thiosulfate 0.5; phenol red 0.024; agar-agar 12.0

Preparation: Sixty five grams of this media was dissolved in one liter of distilled water by heating in a boiling water bath, autoclaved at 121 ^{O}C for 15 minutes, dispensed into test tubes and allowed the medium to solidify to give slant agar tubes.

8. Simmons citrate agar (Difco, Detroit, USA)

Typical composition (g/liter): Magnesium sulfate 0.2; ammonium dihydrogen phosphate 1.0; dipotassium phosphate 1.0; sodium citrate 2.0; sodium chloride 5.0; bacto agar 15.0; bacto brom thymol blue 0.08

Preparation: Twenty four point two (24.2) grams of Simmons citrate agar was suspended in one litre distilled water, boiled to dissolve completely, sterilized at 121°C for 15 minutes and dispensed into test tubes and allowed the medium to solidify to give slant agar tubes.

9. Lysine decarboxylase broth (Difco, Detroit, USA)

Typical composition (g/liter): Bacto peptone 5.0; bacto yeast extract 3.0; bacto dextrose 1.0; L-Lysine 5.0; bacto brom cresol purple 0.02

Preparation: Fourteen grams of the medium was suspended in one liter distilled water, boiled

to dissolve completely and sterilized at 121 ^{O}C for 15 minutes.

10. Urea broth (Merck, Darmstadt, Germany)

Typical composition (g/liter): Yeast extract 0.1; potassium dihydrogen phosphate 9.1; di-sodium hydrogen phosphate 9.5; urea 20.0; phenol red 0.01

Preparation: Thirty eight point five grams urea broth powder was dissolved in one liter of distilled water and sterilized for 5 minutes in a current of steam under mild conditions.

11. MR-VP broth (Methyl-red-Voges-Proskauer broth) (Merck, Darmstadt, Germany)

Typical composition (g/liter): Peptone from meat 7.0; D (+) glucose 5.0; phosphate buffer 5.0

Preparation: Seventeen grams of this medium was dissolved in one litre of distilled water and autoclaved for 15 minutes at 121 ^{O}C. The methyl red indicator solution was also prepared by dissolving 0.04g methyl red in 60ml absolute ethanol.

12. Trypton water (Merck, Darmstadt, Germany)

Typical composition (g/liter): Peptone from casein 10.0; sodium chloride 5.0

Preparation: Fifteen grams of trypton water medium was suspended in one liter of distilled water and autoclaved for 15 minutes at 121°C.

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

Typical 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.

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

Typical 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°C for 15minutes, dispensed into transporting tubes and allowed the medium to solidify.

Appendix II. *Salmonella* isolation in tissue and fecal samples of sheep from Addis Ababa and Modjo abattoirs

Ser. No	Abattoir	Animal code	Types of samples						Total
			Fecal	M. Lymph node	Liver	Spleen	Abdominal muscle	Diaphragmatic muscle	
1	Addis Abeba	S_1	-	-	-	-	-	-	0
2		S_2	-	-	-	-	-	-	0
3		S_3	-	-	-	-	-	-	0
4		S_4	-	-	-	-	-	-	0
5		S_5	-	-	-	-	-	-	0
6		S_6	-	-	-	-	-	-	0
7		S_7	-	-	-	-	-	-	0
8		S8	-	-	-	-	-	-	0
9		S_9	-	-	-	-	-	-	0
10		S_{10}	-	-	-	-	-	-	0
11		S_{11}	+	+	+	+	-	-	4
12		S_{12}	-	-	-	-	-	-	0
13		S_{13}	-	+	-	-	-	-	1
14		S_{14}	-	-	-	-	-	-	0
15		S_{15}	-	-	-	-	-	-	0
16		S_{16}	-	-	-	-	-	-	0
17		S_{17}	-	+	-	-	-	-	1
18		S_{18}	-	-	-	-	-	-	0
19		S_{19}	-	-	-	-	-	-	0
20		S_{20}	-	-	-	-	-	-	0
21		S_{21}	-	-	-	-	-	-	0
22		S_{22}	-	-	-	-	-	-	0
23		S_{23}	-	-	-	-	-	-	0
24		S_{24}	-	-	-	-	-	-	0
25		S_{25}	-	-	-	-	-	-	0
26		S_{26}	-	-	-	-	-	-	0
27		S_{27}	-	-	-	-	-	-	0
28		S_{28}	-	-	-	-	-	-	0
29		S_{29}	-	-	-	-	-	-	0
30		S_{30}	+	-	-	-	-	-	1
31		S_{31}	-	-	-	-	-	-	0
32		S_{32}	-	-	-	-	-	-	0
33		S_{33}	-	-	-	-	-	-	0
34		S_{34}	-	-	-	-	-	-	0
35		S_{35}	-	-	-	-	-	-	0
36		S_{36}	-	-	-	-	-	-	0
37		S_{37}	-	-	-	-	-	-	0
38		S_{38}	-	-	-	-	-	-	0
39		S_{39}	-	-	-	-	+	-	1
40		S_{40}	-	-	-	-	-	-	0
41		S_{41}	-	-	-	-	-	-	0
42		S_{42}	-	-	-	-	-	-	0
43		S_{43}	-	-	-	-	-	-	0
44		S_{44}	-	-	-	-	-	-	0
45		S_{45}	-	-	-	-	-	-	0
46		S_{46}	+	+	-	-	-	-	2
47	Modjo	S_{47}	-	-	-	-	-	-	0
48		S_{48}	-	+	+	-	-	-	2
49		S_{49}	-	-	-	-	-	-	0
50		S_{50}	-	-	-	-	-	-	0
51		S_{51}	-	-	-	-	-	-	0
52		S_{52}	-	-	-	-	-	-	0
53		S_{53}	-	-	-	-	-	-	0

54	Modjo	S_{54}	-	-	-	-	-	-	0
55		S_{55}	-	-	-	-	-	-	0
56		S_{56}	-	-	-	-	-	-	0
57		S_{57}	-	-	-	-	-	-	0
58		S_{58}	-	-	-	-	-	-	0
59		S_{59}	-	-	-	-	-	-	0
60		S_{60}	-	-	-	-	-	-	0
61		S_{61}	-	-	-	-	-	-	0
62		S_{62}	-	-	-	-	-	-	0
63		S_{63}	-	-	-	-	-	-	0
64		S_{64}	-	-	-	-	-	-	0
65		S_{65}	-	-	-	-	-	-	0
66		S_{66}	-	-	-	-	-	-	0
67		S_{67}	-	-	-	-	-	-	0
68		S_{68}	-	-	-	-	-	-	0
69		S_{69}	-	-	-	-	-	-	0
70		S_{70}	-	-	-	-	-	-	0
71		S_{71}	-	-	-	-	-	-	0
72		S_{72}	-	-	-	-	-	-	0
73		S_{73}	-	-	-	-	-	-	0
74		S_{74}	-	-	-	-	-	-	0
75		S_{75}	-	-	-	-	-	-	0
76		S_{76}	-	-	-	-	-	-	0
77		S_{77}		-	-	-	+	-	1
78		S_{78}	-	-	-	-	-	-	0
79		S_{79}	-	-	-	-	-	-	0
80		S_{80}	-	-	-	-	-	-	0
81		S_{81}	-	-	-	-	-	-	0
82		S_{82}	-	-	-	-	-	-	0
83		S_{83}	-	-	-	-	-	-	0
84		S_{84}	-	-	-	-	-	-	0
85		S_{85}	-	-	-	-	-	-	0
86		S_{86}	-	+	-	-	-	-	1
87		S_{87}	-	-	-	-	-	-	0
88		S_{88}	-	-	-	-	-	-	0
89		S_{89}	-	-	-	-	-	-	0
90		S_{90}	-	-	-	-	-	-	0
91		S_{91}	-	-	-	-	-	-	0
92		S_{92}	-	+	-	-	-	-	1
93		S_{93}	+	-	-	-	-	-	1
94		S_{94}	+	+	-	-	-	-	2
95		S_{95}	-	-	-	-	-	-	0
96		S_{96}	-	-	-	-	-	-	0
97		S_{97}	-	-	-	-	-	-	0
98		S_{98}	-	-	-	-	-	-	0
99		S_{99}	-	-	-	-	-	-	0
100		S_{100}	-	-	-	-	-	-	0
101		S_{101}	-	-	-	-	-	-	0
102		S_{102}	-	-	-	-	-	-	0
103		S_{103}	-	-	-	-	-	-	0
104		S_{104}	-	-	-	-	-	-	0

Appendix III. *Salmonella* isolation in tissue and fecal samples of goats from Addis Ababa and Modjo abattoirs

Ser. No	Abattoir	Animal code	Types of samples						Total
			Fecal	M. Lymph node	Liver	Spleen	Abdominal muscle	Diaphragmatic muscle	
1	Addis Ababa	G_{1}	-	-	-	-	-	-	0
2		G_{2}	-	-	-	-	-	-	0
3		G_{3}	-	-	-	-	-	-	0
4		G_{4}	-	-	-	-	-	-	0
5		G_{5}	-	-	-	-	-	-	0
6		G_{6}	-	-	-	-	-	-	0
7		G_{7}	-	-	-	-	-	-	0
8		G8	-	-	-	-	-	-	0
9		G_{9}	-	-	-	-	-	-	0
10		G_{10}	-	-	-	-	-	-	0
11		G_{11}	-	-	-	-	-	-	0
12		G_{12}	-	-	-	-	-	-	0
13		G_{13}	-	-	-	-	-	-	0
14		G_{14}	-	-	-	-	-	-	0
15		G_{15}	-	-	-	-	-	-	0
16		G_{16}	-	-	-	-	-	-	0
17		G_{17}	-	-	-	-	-	-	0
18		G_{18}	-	-	-	-	-	-	0
19		G_{19}	-	-	-	-	-	-	0
20		G_{20}	-	-	-	-	-	-	0
21		G_{21}	-	-	-	-	-	-	0
22		G_{22}	-	-	-	-	-	-	0
23		G_{23}	-	-	-	-	-	-	0
24		G_{24}	-	-	-	-	-	-	0
25		G_{25}	-	-	-	-	-	-	0
26		G_{26}	-	-	-	-	-	-	0
27		G_{27}	-	-	-	-	-	-	0
28		G_{28}	-	-	-	-	-	-	0
29		G_{29}	-	-	-	-	-	-	0
30		G_{30}	-	-	-	-	-	-	0
31		G_{31}	-	-	-	-	-	-	0
32		G_{32}		-	-	-	-	-	0
33		G_{33}	-	-	-	-	-	-	0
34		G_{34}	-	-	-	-	-	-	0
35		G_{35}	-	-	-	-	-	-	0
36		G_{36}	-	-	-	-	-	-	0
37		G_{37}	+	-	-	-	-	-	1
38		G_{38}	-	-	-	-	-	-	0
39		G_{39}	-	-	-	-	-	-	0
40		G_{40}	-	-	-	-	-	-	0
41		G_{41}	-	-	-	-	-	-	0
42		G_{42}	-	-	-	-	-	-	0
43		G_{43}	-	-	-	-	-	-	0
44		G_{44}	-	-	-	-	-	-	0
45		G_{45}	-	-	-	-	-	-	0
46	Modjo	G_{46}	-	-	-	-	-	-	0
47		G_{47}	-	-	-	-	-	-	0
48		G_{48}	-	-	-	-	-	-	0
49		G_{49}	+	+	-	-	-	-	2
50		G_{50}	-	-	-	-	-	-	0
51		G_{51}	-	-	-	-	-	-	0
52		G_{52}	-	-	-	-	-	-	0
53		G_{53}	-	-	-	-	-	-	0

54	Modjo	G_{54}	-	-	-	-	-	-	0
55		G_{55}	-	-	-	-	-	-	0
56		G_{56}	-	-	-	-	-	-	0
57		G_{57}	-	-	-	-	-	-	0
58		G_{58}	-	-	-	-	-	-	0
59		G_{59}	-	-	-	-	-	-	0
60		G_{60}	-	-	-	-	-	-	0
61		G_{61}	-	-	-	-	-	-	0
62		G_{62}	-	-	-	-	-	-	0
63		G_{63}	-	-	-	-	-	-	0
64		G_{64}	-	-	-	-	-	-	0
65		G_{65}	-	-	-	-	-	-	0
66		G_{66}	-	-	-	-	-	-	0
67		G_{67}	-	-	-	-	-	-	0
68		G_{68}	-	-	-	-	-	-	0
69		G_{69}	-	-	-	-	-	-	0
70		G_{70}	-	-	-	-	-	-	0
71		G_{71}	-	-	-	-	-	-	0
72		G_{72}	-	-	-	-	-	-	0
73		G_{73}	-	-	-	-	-	-	0
74		G_{74}	-	-	-	-	-	-	0
75		G_{75}	-	-	-	-	-	-	0
76		G_{76}	-	-	-	-	-	-	0
77		G_{77}	-	-	-	-	-	-	0
78		G_{78}	-	-	-	-	-	-	0
79		G_{79}	-	-	-	-	-	-	0
80		G_{80}	-	-	-	-	-	-	0
81		G_{81}	-	+	-	-	-	-	1
82		G_{82}	-	-	-	-	-	-	0
83		G_{83}	-	-	-	-	-	-	0
84		G_{84}	-	-	-	-	-	-	0
85		G_{85}	-	-	-	-	-	-	0
86		G_{86}	-	-	-	-	-	-	0
87		G_{87}	-	-	-	-	-	-	0
88		G_{88}	-	-	-	-	-	-	0
89		G_{89}	-	-	-	-	-	-	0
90		G_{90}	-	-	-	-	-	-	0
91		G_{91}	-	-	-	-	-	-	0
92		G_{92}	-	-	-	-	-	-	0
93		G_{93}	-	-	-	-	-	-	0
94		G_{94}	-	-	-	-	-	-	0
95		G_{95}	-	-	-	-	-	-	0
96		G_{96}	-	-	-	-	-	-	0
97		G_{97}	-	-	-	-	-	-	0
98		G_{98}	-	-	-	-	-	-	0
99		G_{99}	-	-	-	-	-	-	0
100		G_{100}	-	-	-	-	-	-	0

9. CURRICULUM VITAE

A. Biographical Data:

Name	Wassie Molla
Date of birth	January 7, 1970
Place of birth	Estie, South Gondar, Ethiopia
Marital status	Married
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Research officer in regional laboratory, Kombolcha

B. Educational background

1977/78-1981/82	Elementary School Astedemariam Elementary School, Estie, South Gondar Shimegiorgis Elementary School, Estie, South Gondar
1982/83-1987/88	Junior Secondary School and High School Mekane-Eyesus High School, Estie, South Gondar, Ethiopia Achievement: Ethiopian School Leaving Certificate Examination
1988/89-1994/95	University/Under graduate program Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia Achievement: Doctor of Veterinary Medicine, D.V.M. Degree
2002/03-2003/04	Postgraduate study Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia Achievement: MSc in Tropical Veterinary Medicine

C. Work Experience

March, 1996-may, 1996	Community development project facilitator, Finish International Development, Lalibela
June, 1996-Dec., 1996	District veterinary officer, District Agricultural office,

	Delanta Dawint woreda, North Wollo
Jan., 1997-Dec., 2000	Zonal veterinary officer, Zonal Department of Agriculture, North Wollo
Jan., 2001-Sep., 2002	Research officer, Kombolcha Regional Veterinary Laboratory

D. Research out put/Technical paper

a. Trace mineral nutrition for livestock in Ethiopia (Seminar paper, 1993)
b. Prevalence of bovine and ovine fascioliasis: A preliminary survey in Nekemte and its surrounding areas (DVM thesis, 1995)
c. Salmonellosis in apparently healthy slaughtered sheep and goats in Addis Ababa and Modjo abattoirs (MSc thesis, 2004)

E. Membership

Member of the Ethiopian Veterinary Association

F. Language

Amharic	Mother Tongue
English	Writing and speaking

G. Computer Skill

MS Dose
Microsoft Word
Microsoft Excel
Microsoft Access