ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

SALMONELLA SEROVARS IN APPARENTLY HEALTHY SLAUGHTERED CATTLE AT BAHIR DAR ABATTOIR, ETHIOPIA

BY

SEFINEW ALEMU

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DEBRE ZEIT, ETHIOPIA

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SALMONELLA SEROVARS IN APPARENTLY HEALTHY SLAUGHTERED CATTLE AT BAHIR DAR ABATTOIR, ETHIOPIA

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical Veterinary Microbiology

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LIST OF ABBREVIATIONS

AFSSA	Agence Française de Securite Sanitaire des Aliments
BPW	Buffered Peptone Water
CDC	Center for Disease Control
CS	Carcass swab
DNA	Deoxyribonucleic acid
H	Flagellar antigen
H_2S	Hydrogen sulphide
HACCP	Hazard Analysis Critical Control Point
IC	Intestinal content
ISO	International Organization for Standardization
LIA	Lysine iron agar
LPS	Lipopolysaccharide
MKTTn	Muller-Kauffmann tetrathionate novobiocin broth
MLN	Mesenteric lymph nodes
NCCLS	National Committee for Clinical Laboratory Standards
O	Somatic antigen
OIE	Office International des Epizooties
PCR	Polymerase Chain Reaction
RVS	Rappaport-Vassiliadis medium with Soya
TSB	Trypticase Soy Broth
TSI	Triple sugar iron agar
Vi	Capsular antigen
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate agar

ABSTRACT

The present cross-sectional study was undertaken to determine the prevalence, distribution and antimicrobial resistance patterns of *Salmonella* serovars from apparently healthy slaughtered cattle from October, 2006 to March, 2007 at Bahir Dar abattoir, Ethiopia. A total of 744 samples consisting of liver, mesenteric lymph nodes, intestinal content and carcass swab (each n = 186) were collected from 186 slaughtered cattle. Bacteriological analysis for *Salmonella* was done following the recommendations of the International Organization for Standardization (ISO 6579, 2002). Serotyping of the *Salmonella* isolates was done at Agence Française de Securite Sanitaire des Aliments (AFSSA), Cedex, France.

Salmonella was isolated in one or more of liver, mesenteric lymph nodes, and intestinal content from 13 (7%) of the slaughtered cattle. Of the 744 samples analyzed, 28 (3.8%) were positive for *Salmonella.* There was difference in the prevalence of *Salmonella* among liver, mesenteric lymph nodes, intestinal content and carcass swab samples; difference in prevalence was not statistically significant (Fisher's exact = 0.06, $P > 0.05$). The highest prevalence 5.9% (11 of 186) was detected from intestinal content while the lowest prevalence 1.1% (2 of 186) was detected from liver. The prevalence of *Salmonella* in mesenteric lymph nodes was 3.2% (6 of 186). Of the total 186 examined animals, carcass contamination was observed in 4.8% (9 of 186) of them. However, there was no statistical association between the prevalence of carcass contamination and the prevalence of any of the other samples.

Six different serovars consisting of *Salmonella* Typhimurium, *S.* Newport, *S.* Haifa, *S.* Heidelberg, *S.* Infantis and *S.* Mishmarhaemek were identified among the 28 isolates. *Salmonella* Typhimurium and *S.* Newport were the most frequently isolated serovars each contributing 21.4% (6 of 28) of the total isolates while *Salmonella* Heidelberg and *S.* Mishmarhaemek were the least frequently isolated, each with a prevalence of 7.1% (2 of 28).

Antimicrobial resistance test was done for all the 28 isolates against eight antimicrobials by disk diffusion technique based on the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997). Among the 28 isolated *Salmonella* 39.3% (11 of 28) were resistant to one or more of the tested antimicrobials, of which 63.6% (7 of 11) were resistant to a single antimicrobial while the rest 36.4% (4 of 11) were resistant to two or more of the antimicrobials (multiple antimicrobial resistant). All of the isolates were sensitive to the

antimicrobial effects of gentamycin, norfloxacin, and trimethoprim. Eleven of 28 (39.3%) of the isolates were resistant to streptomycin followed by tetracycline and ampicillin with prevalence of 14.3% (4 of 28) and 7.1% (2 of 28) respectively. The identified serovars had different antimicrobial resistance patterns. In *Salmonella* Haifa 66.7% of them were multiple antimicrobial resistant to streptomycin, ampicillin and tetracycline while the rest 33.3% were resistant only to streptomycin. All isolates of *Salmonella* Typhimurium were susceptible to the tested antimicrobials except one isolate which was resistant to chloramphenicol, streptomycin and tetracycline. *Salmonella* Haifa was multiple antimicrobial resistant to ampicillin, tetracycline and streptomycin. *Salmonella* Mishmarhaemek and *S.* Infantis were susceptible to all of the tested antimicrobials while all serovars of *S.* Heidelberg were resistant to streptomycin.

In conclusion, there is possibility of the public to be at risk due to high level of carcass contamination with the single and/or multiple antimicrobial resistant *Salmonella* serovars observed in this study. Therefore, hygienic slaughtering in the abattoir and proper cooking of meat at household level should be practiced. The consumer should be aware of the risk of consumption of raw or undercooked meat under the meat shed of Bahir Dar abattoir.

Key words: Cattle, *Salmonella*, Serovars, Antimicrobials, Contamination, Bahir Dar, Ethiopia.

1. INTRODUCTION

Salmonellosis is a collective description of a group of diseases caused by bacteria of the genus *Salmonella* with signs that vary from severe enteric fever to mild food poisoning (Jones *et al*., 2004). Salmonellosis has a wide variety of domestic and wild animal hosts (Acha and Szyfres, 2001) and has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry (Wray and Davies, 2003). The situation of salmonellosis is usually difficult to evaluate in developing countries where there are very limited scope of studies and lack of coordinated epidemiological surveillance system. However, epidemic outbreaks are known to occur in these countries (Acha and Szyfres, 2001).

Salmonellosis is an important disease both in human and animals (Radostits *et al.*, 1994; OIE, 1996) and is the leading most common foodborne zoonoses with worldwide distribution (D'Aoust, 1997; Acha and Szyfres, 2001; Wray and Davies, 2003; Maddox, 2003). However, the incidence seems to vary between countries (Acha and Szyfres, 2001). Infection in animals is of importance because of the direct economic consequences of salmonellosis attributable to mortality and morbidity. Of even greater importance are the human health consequences of salmonellosis acquired by direct or indirect contact with animals, which constitute a vast reservoir of these organisms (Libby *et al.*, 2004).

As a zoonosis, the disease has assumed increasing importance in recent years because of the much more frequent occurrence of human salmonellosis, with animal salmonellosis as the principal reservoir (Radostits *et al*., 1994). The economic losses associated with human salmonellosis are not only associated with investigations, treatment, and prevention of illness but also affect the whole human chain of food production (Radostits *et al*., 1994; Wray and Davies, 2003).

The distribution and importance of serovars involved in salmonellosis in human and animals vary over time for usually quite unknown reasons (Radostits *et al.*, 1994; Lax *et al*., 1995; D'Aoust, 1997; Wray and Davies, 2003). Currently it is impossible to predict when and why a serovar will decline or prominence, either as a result of natural causes or control measures, and when new serovar is likely to rise in importance, and whether it will

be a greater or lesser threat to animal and human health. This highlights the importance of research (Lax *et al*., 1995).

The ubiquitous distribution of *Salmonella* in the natural environment and its prevalence in the global food chain, the physiological adaptability and its potentially serious economic impact on the food chain industry predicate the need for continued attention and stringent controls at all levels of food production (D'Aoust, 1994). In human, the source of the large majority of non-thyphoidial salmonellosis is food of animal origin (Acha and Szyfres, 2001). Therefore understanding of salmonellosis and the eventual solution of the current disease problems may only arise from detailed knowledge of epidemiology of the organism and an interrelated study of the disease in all groups of animals to develop a detailed control program (Wray and Davies, 2003; Jones *et al*., 2004).

In *Salmonella*, increasing levels of drug resistance from *Salmonella* recovered animals is of considerable concern (Libby *et al.*, 2004). The most serious risk of salmonellosis in human is that when animals from which they originate have been treated with the particular antimicrobials over a long period, transmitted bacteria will have acquired resistance to specific antimicrobials. Therefore in addition to concern about the presence of *Salmonella* species as a potential foodborne pathogen, concern has also been raised about the human health impact of presence of genetic determinants for antimicrobial resistance transferred among these organisms (Dargatz *et al*., 2003), which limits therapeutic options for treatment of disease in human and animals (Radostits *et al*., 1994; Gebreyes *et al*., 2000). These concerns have resulted in intense efforts in many countries to substantially reduce the level of infection in animals (Libby *et al.*, 2004).

The study of shift in susceptibility of *Salmonella* organisms to various antibacterial agents has also become necessary for effective treatment and prediction of occurrence of resistant populations of prevalent serovars (Bax *et al*., 2001; Esaki *et al*., 2004). It allows to formulate strategies aimed at reducing or preventing any further development of resistance (Bax *et al*., 2001), or in the development of antimicrobial analogs that will be useful in the treatment of drug resistant organisms (Mills- Robertson *et al*., 2003). This requires of special considerations particularly in developing countries like Ethiopia where antimicrobials are indiscriminately used and their effectiveness against common human and animal bacterial pathogens is rarely determined (Molla *et al*., 1999).

Salmonellosis is a significant disease of ruminants, mainly cattle (Hirsh, 1999; Jones *et al*., 2004) and surge of prevalence has been seen due to intensification of husbandry (Radostits *et al.*, 1994). Studies conducted in slaughtered cattle and cattle products in Ethiopia indicated a number of *Salmonella* serovars (Pegram *et al*., 1981; Abdella *et al*., 1996; Tegegne and Ashenafi, 1998; Nyeleti *et al*., 2000; Alemayehu *et al*., 2003; Ejeta *et al*., 2004; Zewdu, 2004; Sibhat, 2006). Salmonellae are wide spread in slaughter cattle and a possible linkage exists between cattle and human isolates in Addis Ababa and Debre Zeit, Ethiopia which require further detailed epidemiological and molecular studies (Nyeleti *et al*., 2000; Alemayehu *et al*., 2003; Sibhat, 2006). The occurrence of *Salmonella* serovars in cattle is a problem for both food safety, animal health, and as a source of human infection (Rabsch *et al*., 2003). In Ethiopia, it was also observed that *Salmonella* was one of the major causes of diarrhea in human (Gedebou and Tassew, 1981; Ashenafi and Gedebou, 1985; Mache *et al*., 1997; Mache, 2002).

Therefore, there is need of epidemiological study, denoting prevalent *Salmonella* serovars, and antimicrobial resistance patterns in food animals and human in Ethiopia to control salmonellosis, or at least to formulate a detailed control program. Hence this cross-sectional study was designed to study salmonellosis in apparently healthy slaughtered cattle at Bahir Dar municipality abattoir as part of generating base line information with the following objectives:

- To estimate the prevalence and distribution of *Salmonella* in slaughtered cattle,
- To find out the diversity of *Salmonella* serovars, and
- To determine the antimicrobial resistance pattern of *Salmonella* serovars isolated from slaughtered cattle at Bahir Dar abattoir.

2. LITERATURE REVIEW

2.1. The Genus *Salmonella*

Salmonella are facultative anaerobic, non spore forming, Gram-negative rods within the family *Enterobacteriaceae*. All but two (*Salmonella* Pullorum and *S*. Gallinarum) are motile by means of peritrichous flagella. *Salmonella* are non-lactose fermenting, with the exception of *S. enterica* subspecies *arizonae* and *diarizonae* (Libby *et al*., 2004).

Salmonella grows in temperature range of 2 - 47°C with rapid growth between 25 and 43°C (D'Aoust, 2001) and pH range of 4 to 8, with an optimum temperature of 37°C and optimum pH of 6.5 to 7.5 but cannot survive at temperatures higher than 70°C. The microorganisms in this genus readily adapt to extreme environmental conditions (D'Aoust, 1997; Acha and Szyfres, 2001). These bacteria can resist dehydration for a very long time, both in feces and in foods (Acha and Szyfres, 2001).

2.1.1. Taxonomy and nomenclature

The genus *Salmonella* obtained its name from the American veterinarian Daniel E. Salmon, who first isolated *S*. enterica serovar Choleraesuis from swine in 1885 (Rabsch *et al*., 2003). The genus encompasses a large taxonomic group (Heyndrickx *et al*., 2005). *Salmonella* nomenclature is complex, and scientists use different systems to refer to and communicate about this genus (Brenner *et al*., 2000). Unresolved issues include the name of the type species and the system of naming members of the genus (Libby *et al*., 2004). However, uniformity in *Salmonella* nomenclature is necessary for communication between scientists, health officials, and the public (Brenner *et al*., 2000).

A decision of the ''Judicial Commission of the International Committee for Systematics of Prokaryotes'' was recently issued in an endeavour to resolve the discrepancy between the widely accepted nomenclatural system and the system conforming to the Bacteriological Code. According to this decision, the type species of the genus *Salmonella* is now *Salmonella enterica* instead of *Salmonella choleraesuis* (Judicial Commission, 2005). It

was also decided that the epithet enterica in *Salmonella* enterica is to be preferred over all earlier epithets that may be applied to this species.

The real consequences of this ruling may not however be apparent to all bacteriologists. The decision means that the use of the species name *Salmonella* enterica (and of its subsequent subspecies enterica) is now in conformity with the Bacteriological Code, but does not imply that the species names *Salmonella* choleraesuis and other valid species names have to be abolished from now on. It is only hoped that by this action the use of the latter names will further diminish in favor of the preferred species name *Salmonella* enterica (Heyndrickx *et al*., 2005).

The nomenclature of *Salmonella* is still evolving and there is the debate on the name for the type species (Brenner *et al*., 2000). It is a serious problem in trying to reach international agreement on a complex and expanding genus (Heyndrickx *et al*., 2005), therefore it is unlikely to be settled any time soon (Brenner *et al*., 2000).

At present, the scheme conceived by Kauffmann-White is used (Acha and Szyfres, 2001). In a modification of this scheme, two species are proposed by Le Minor and Popoff (1987) of the WHO Collaborative Center for Reference and Research on *Salmonella* including Center for Disease Control (CDC) (Brenner *et al*., 2000). According to the scheme proposed by Le Minor and Poppof (1987), the genus *Salmonella* consists of two species: *S*. enterica and *S*. bongori (formerly called *S.* enterica subsp. bongori) (D'Aoust, 2001; Quinn *et al*., 2002). *Salmonella* enterica is divided into six subspecies: *S*. enterica subsp. enterica, *S*. enterica subsp. salamae, *S*. enterica subsp. arizonae, *S*. enterica subsp. diarizonae, *S*. enterica subsp. houtenae and *S*. enterica subsp. Indica (Quinn *et al*., 2002).

The names given to the serovars do not follow the usual rules of nomenclature. The first serovars to be identified were given names that indicate the disease with which they were associated or their common animal host, and these names become accepted by clinical microbiologists continue in common use. Serovars isolated subsequently are named after the town or region in which they were first isolated, or are designated solely by their antigenic formulae (Jones *et al*., 2004).

Historically, serovars of *Salmonella* were considered as species and, for this reason, the serovar names were italicized. Therefore it is no longer seemed justified to consider serovar names as species names. In practice, the individual serovar names are retained, but preferably they should now be indicated with an initial capitalized letter and not italicized (Heyndrickx *et al*., 2005). They are in fact without taxonomic status, used to name bacteria. In clinical practice, the subspecies name does not need to be indicated as only serovars of subspecies enterica bear a name. The name *Salmonella* serovar Typhimurium or *Salmonella* Typhimurium may be used for routine practice. Serovars of other subspecies of S. enterica and those of S. bongori are designated only by their antigenic formula (Popoff and Le Minor, 1997). The species name is usually S. *enterica* (sometimes subsp. e*nterica*) serovar typhimurium or S. *enterica* serovar dublin (Jones *et al*., 2004).

For epidemiological purposes, the salmonellae can be placed in to three groups (Jay, 2000). *Salmonella* serovars, which are almost exclusively associated with one particular host species, referred as host-restricted serovars. Serovars, which are prevalent in one particular host species but can also cause disease in other host species, referred as host-adapted serovars and ubiquitous serovars to be referred as un-restricted serovars (Uzzau *et al*. 2000).

2.2. Epidemiology

The epidemiology of salmonellosis as a disease of animals and zoonosis is complex (Radostits *et al*., 1994; Wray and Davies, 2003). 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). The complexity of epidemiology and the many routes by which the organism can be transmitted presents a major challenge to all involved in animal production and controlling *Salmonella* (Wray and Davies, 2003).

2.2.1. Distribution

Members of the genus *Salmonella* are ubiquitous pathogens found in human and their livestock, wild mammals, reptiles, birds, and even insects (Davis *et al*., 1990) and their primary habitat is the intestinal tract of these animals, however they may be found in other parts of the body from time to time (Wray and Davies, 2003).

The distribution and importance of serovars involved in salmonellosis in human and animals vary over time for usually quite unknown reasons (Radostits *et al*., 1994; Lax *et al*., 1995; D' Aoust, 1997; Wray and Davies, 2003). Some serovars maintain their dominant role over many years, others, emerge, or re-emerge or decrease over time (D' Aoust, 1997). Therefore, a future of *Salmonella* infections in all species is a continual fluctuation in the proportion of the serovars involved. It is common for a serovar to be introduced to a country and to establish in one or more species, possibly as the predominant serovar and then to decline without any apparent reason or without intervention of public health or veterinary authorities (Jones *et al*., 2004).

As intestinal forms, the organisms are excreted in feces from which may be transmitted by insects and other living creatures to a large number of places including water, especially polluted water (Jay, 2000) human sewage, and in any material subject to fecal contamination (Wray and Davies, 2003).

2.2.2. Host range

Salmonellae have a wide variety of domestic and wild animal hosts (Acha and Szyfres, 2001). The vast majority of serovars of *Salmonella enterica* show no host adaptation (Libby *et al.,* 2004). However, some serovars appear to show a degree of host adaptation and primarily infect one animal species. For example, *S*. Pullorum and *S*. Gallinarum infect poultry, *S*. Choleraesuis occurs in swine, and *S*. Dublin appears to have predilection for cattle, although occasional outbreaks of disease caused by this serovar occur in sheep (Wray and Davies, 2003). In contrast, *S*. Typhimurium and *S*. Enteritidis are frequently isolated from a variety of vertebrates with and without clinical disease and may be

considered the least host-adapted serovars. Typically, host adapted serovars cause sever systemic disease in adult as well as young hosts, where as the unadapted serovars are associated with enteric disease primarily in young hosts (Libby *et al.,* 2004).

2.2.3. Sources of infection and modes of transmission

Infected animals, which they excrete and infect other animals directly or indirectly by contamination of the environment primarily feed and water supplies are the sources of the organisms (Radostits *et al*., 1994; Acha and Szyfres, 2001). Feed is a major potential route by which new infections may be introduced into a herd or flock (Davies and Hinton, 2000). Many new serovars have been introduced into countries in imported feed ingredients (Clark *et al.,* 1973). Hay and basic cereal ingredients such as barley or maize can be contaminated during storage, by wild animals, especially rodents or birds but not considered particularly susceptible to *Salmonella* (Glickman *et al*., 1981). Contaminated drinking water may facilitate the rapid spread of *Salmonella* among farm animals, which often defecate in their drinking water. Contamination may occasionally occur in the water storage tanks in a building, from wild animal feces or even from carcasses. Animals may be infected either by drinking such water or from contamination of the pasture when flooding occurs (Williams, 1975). Most infections are introduced into *Salmonella*-free herds by the purchase of infected animals for intensive rearing or adult animals for replacements. Purchased animals may have acquired infection on their home-farm premises, on transit or on dealers' premises (Wray *et al*., 1991).

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

Any food of animal origin can be a source of infection for human particularly contaminated poultry, pork, beef, eggs, milk, and milk and egg products are the most common vehicles. Chickens, turkeys, geese, and ducks constitute the most important animal reservoir of asymptomatic *Salmonella* excretors in the human food chain (D'Aoust, 2001; Acha and Szyfres, 2001). Meat can become contaminated in abattoirs by means of contaminated equipment and utensils during skinning and butchering (Acha and Szyfres, 2001).

Foods of vegetable origin contaminated by animal products, human excreta, or dirty utensils, in both commercial processing plants and household kitchens, have occasionally been implicated as vehicle of human salmonellosis. Contaminated public or private water supplies are important sources of infection in typhoid fever (caused by *S*. Typhi) and, less frequently, in other *Salmonella* infections (Acha and Szyfres, 2001). Transmission of *Salmonella* species to human by direct or indirect contact to animals has also been reported (Fey *et al*., 2000**;** Hendriksen *et al*., 2004)**.**

2.2.4. Carrier state

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

When an animal is infected with *Salmonella* it may become a clinical case or an active carrier, passing organisms constantly or intermittently in the feces. It may also become a latent carrier with infection persisting in lymph nodes or tonsils but no salmonellae in the feces, or a passive carrier which is constantly picking up infection from pasture or the calf pen floor but the infection disappears when it is removed from the environment. These animals probably multiply the salmonellae without becoming permanent carriers (Radostits *et al*., 1994; Jones *et al*., 2004). The importance of the latent carriers is that they can become active carriers or even clinical cases under stress, especially at calving time. Thus

persistence of infection in animals and in the environment is important epidemiological features of salmonellosis (Radostits *et al*., 1994).

Identification of carriers by bacteriological examination is more difficult and an attempt must be made to distinguish active carriers (persistent excretors) from passive carriers and latent carriers (intermittent excretors) (Jones *et al*., 2004) and animals should not be assumed to be excretors based on one isolation, nor should they be considered to be free of infection unless at least three negative fecal samples have been obtained. Even in the latter case latent carriers and animals that will eventually clear the infection, and yet are still harboring salmonellosis will be missed. Therefore, latent carriers of the organism, unlike persistent excretors, cannot be readily identified by fecal culture or serological methods (Jones *et al*., 2004).

Treatment does not eliminate carriers; rather it promotes the carrier state and resistant strains, although might control mortality (Hirsh, 1999). In human, carrier state are of concern to the food manufacturing and food service industries because of the perceived risk of cross-contamination of food by infected food handlers, and potentiation of foodborne disease outbreaks (D'Aoust, 1991).

2.3. Predisposing factors to clinical salmonellosis

There are risk factors related to the host, the environment and the pathogen, which affect the development of salmonellosis. Except in the newborn, infection with *Salmonella* is not a single cause of salmonellosis and the response to infection varies depending on the size of the challenge dose, the immunological status of the animal and exposure to stressors particularly in older animals (Radostits *et al*., 1994).

If the infection pre-exists, any significant change in management of the herd or group of animals can precipitate the onset of clinical salmonellosis (Radostits *et al*., 1994). Factors which predispose animals to salmonellosis and precipitate clinical disease in infected cattle and the excretion of the bacteria by carrier animals include unhygienic conditions, overcrowding, transportation, insufficient intake of colostrum, inadequate availability of water and feed, inclement weather, hospitalization, surgery and parturition. Intercurrent

infections that may play a role include bovine virus diarrhea and mucosal disease, infectious bovine rhinotracheitis and *E. coli,* or infestation with parasites such as *Fasciola hepatica.* The effects of these 'stress factors' are probably related to the suppression of cell-mediated immunity (Venter *et al.,* 1994).

Disruption of the normal intestinal flora by factors such as antimicrobial therapy, diet, and water deprivation increases the hosts' susceptibility to infection. Reduced peristalsis, stress due to transportation and overcrowding also predispose to the colonization of the intestine by salmonellae (Quinn *et al*., 1999; Bhatia and Ichhpujani, 1994).

Gastric acidity is responsible for eliminating a large proportion of ingested organisms in both monogastric animals and ruminants. In ruminants, survival of salmonellae is further reduced by the high concentration of short chain fatty acids in rumen fluid and consequently salmonellae disappear rapidly from the rumen of regularly fed cattle. Factors which alter the acidity and composition of rumen fluid, such as starvation and refeeding increase susceptibility to infection. The bacterial flora of intestines may also be inhibitory to infection and the absence of fully developed intestinal flora may in part account for the comparative lack of resistance of young animals to salmonellosis (Jones *et al*., 2004). Thus adequate nutrition prior to and during transport and with in the abattoir could be an important intervention to prevent spread of *Salmonella* (Wray and Davies, 2003).

2.4. Pathogenesis

Salmonella commonly enter the host through ingestion, although inhalation may be an important route in swine. Following ingestion, the organism must survive exposure to the low $_{P}$H of the stomach. Despite having a number of systems to resist acidity, only a small percentage of ingested salmonellae usually survive exposure to the low $_{P}$H and move into the small intestine (Libby *et al*., 2004). Salmonellae need to colonize the distal small intestine or colon to initiate enteric disease (Quinn *et al*., 1999) and localize in the mucosae of the ileum, caecum and colon, and in the mesenteric lymph nodes of infected animals (Quinn *et al*., 2002). The main initial site of entry is probably the distal ileum. This may be because the intestinal contents are held within the distal ileum for some time before entry into the caecum and it may also be partly due to the relatively high number of M cells

(specialized epithelial cells overlaying the payer's patches) in the distal ileum (Jones *et al*., 2004).

In the intestine *Salmonella* encounters protective antibacterial factors and mechanisms such as bile salts, intestinal mucus, lysozyme, lactoferrin, peristalysis, and organic acids. In the large intestine, the abundant and complex established normal flora is a major obstacle to colonization. Factors that impair these host defenses will predispose to intestinal colonization and disease (Libby *et al*., 2004).

The virulence of *Salmonella* relates to their ability to invade host cells, replicates in them and resists both digestions by phagocytes and destruction by the complement components of the plasma. Following adherence, probably through fimbrial attachment, to the surface of intestinal mucosal cells, the bacteria induce ruffling of cell membranes (Quinn *et al*., 2002). Some strains producing enteritis and diarrhea appear capable of forming LT-like and ST-like enterotoxins and cytotoxins. However, diarrhea due to salmonellosis is thought to be associated primarily with the inflammatory response, which stimulates local prostaglandin synthesis (Quinn *et al*., 1999).

The invasive strains replicate intracellularly but cause little mucosal damage or inflammation. They pass rapidly through the epithelial barrier and eventually proliferate in the lamina propria; they multiply within the macrophage of the liver and spleen as well as intravascularly. Any salmonellae in non-immune animals that are phagocytosed tend to survive within the phagocyte. Multiplication of the organisms in the body leads to a severe endotoxaemia. Invasive strains occur frequently in *S.* Typhi, *S.* Dublin and *S*. Typhimurium (Davis *et al*., 1990; Quinn *et al*., 1999).

Local host defense mechanisms prevent access of the invading *Salmonella* to the lymphatic and systemic circulation, so that infection with nontyphoidal strains is usually mild and self-limiting. However, serious sequel, and even death, may occur in hosts impaired in humoral or in cell-mediated immunity or with a compromised reticuloendothelial system. The acute gastroenteritis caused by many *Salmonella* serovars is also associated with transient bacteriamia (Davis *et al*., 1990).

2.5. Clinical forms of salmonellosis

In any discussion about salmonellosis in large animals there is likely to be a significant difference of opinion about its clinical behavior (Radostits *et al*., 1994). Infection by *Salmonella* may or may not be clinically apparent. In subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material briefly, intermittently, or persistently (Acha and Szyfres, 2001). The disease is most satisfactorily described as three syndromes classified arbitrarily according to severity as septicemia, acute enteritis and chronic enteritis (Radostits *et al*., 1994).

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

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

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

In human, the clinical pattern of salmonellosis can be divided into gastroenteritis, enteric fever (typhoid-like disease), bacteriamia with or without focal extraintestinal infection, and the asymptomatic carrier state. Virtually any *Salmonella* serovar can cause any of these manifestations under appropriate conditions (e.g. in an immunocompromised host) and can persist afterwards. However, certain serovars are likely to be associated with a particular clinical syndrome: for example, *S*. Typhimurium, *S*. Enteritidis, and *S*. Newport with gastroenteritis: *S*. Typhi and the paratyphoid serovars with enteric fever; and *S*. Choleraesuis with bacteriamia and focal infection without antecedent gastrointestinal disturbance (Davis *et al*., 1990).

2.6. Detection of *Salmonella*

The clinical signs and findings at post-mortem examination are not unique to salmonellosis and although a tentative diagnosis may be made this should be confirmed in diseased animals or at necropsy by isolation of the organism (Jones *et al*., 2004). Numerous culture protocols have been developed and modified to reliably recover and characterize *Salmonella* species from a broad range of sources. On-farm hazard analysis critical control point (HACCP) approaches for improving food safety and animal health have resulted in attempts to optimize antemortem, postmortem and environmental specimen collection, processing, and culture for *Salmonella* detection (Maddox, 2003).

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

Recent emphasis on food safety issues and the development of guidelines using *Salmonella* as an indicator for enteric contamination of meats and potential pathogen in animal products has led to efforts to standardize detection techniques (Maddox, 2003). Standard

culture methods for the detection of foodborne *Salmonella* serovars generally consist of pre-enrichment in non selective liquid medium, enrichment in selective liquid media, plating on differential agar, biochemical screening, and serological confirmation of identity (D'Aoust, 2001; ISO 6579, 2002).

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

2.6.1. Pre-enrichment in non-selective liquid medium

Salmonellae may be present in small numbers in a particular mass or volume of product and are often accompanied by considerably larger numbers of other *Enterobacteriaceae* or other families or it may be sublethally injured (ISO 6579, 2002). For example, the number of salmonellae in feces from asymptomatic animals, environmental samples, animal feed and food is usually low (Andrews, 1989; D'Aoust, 2001) and may not be detected by conventional culture techniques (Davies and Hinton, 2000; Wray and Davies, 2003). In their study in "ayib" (Ethiopian cottage cheese) Abdella and his colleagues (1996) reported that a higher recovery of *Salmonella* has been observed in all methods involving a pre-enrichment procedure. Therefore a test portion is initially inoculated into nutritious non-inhibitory pre-enrichment liquid medium to favor the small number of salmonellae, which may otherwise be killed by the toxic effect of enrichment media, to multiply to detectable levels or it may help to resuscitate salmonellae that have been stressed or sub lethally-injured arising from exposure to heat, freezing, desiccation, preservatives, high osmotic pressure or wide temperature fluctuations (Andrews, 1989; D'Aoust, 2001; Doyle and Cliver, 1990; ISO 6579, 2002).

Generally, pre-enrichment media are nutritionally complex (Doyle and Cliver, 1990). Traditional pre-enrichment media include non-fat dry milk with added brilliant green dye for the pre-enrichment of cocoa and chocolate products, brilliant green water for milk powder, tripticase soy broth supplemented with potassium sulfite to neutralize spice-dependent bacteriostasis, nutrient broth, or lactose milk (Doyle and Cliver, 1990; D'Aoust, 2001), and buffered peptone water (D'Aoust, 2001, ISO 6579, 2002).

2.6.2. Enrichment in selective liquid media

Enrichment of *Salmonella* can have profound effects on the ability to recover them from animal sources, foods, water or environmental samples. The method selected will vary with regard to the need for non-selective nutrient broths such as buffered peptone water (BPW) or trypticase soy broth (TSB) for resuscitating damaged cells from heat-stressed, desiccated, or otherwise less than ideal samples. Other specimens containing high numbers of competing bacteria such as feces or ground meats may require highly selective media to prevent overgrowth by coliforms that can readily out-compete the *Salmonella*. Incubation time and temperature also will influence the success of the enrichment process based upon the type and source of the specimen cultured (Maddox, 2003).

In general a large number of liquid enrichment media are necessary to guarantee the isolation of the majority of serovars. Enrichment media depend upon allowing salmonellae to grow, although some times in an inhibited manner, whilst suppressing other bacteria through the action of chemicals, dyes, antimicrobials and enhanced incubation temperature. In common use are media containing sodium selenite or tetrathionate and dyes such as brilliant green or malachite green to which salmonellae are relatively resistant (Jones *et al.*, 2004).

2.6.3. Plating out and identification

To isolate *Salmonella*, enrichment cultures are plated onto selective agar plates (Doyle and Cliver, 1990; Jones *et al*, 2004). The use of at least two types of solid isolation media is necessary to guarantee the isolation of the majority of serovars (Jones *et al*, 2004).

Examples of selective plating agars include Hektoen enteric, xylose-lysine-deoxycholate (XLD), bismuth sulfite, MacConkey (Doyle and Cliver, 1990; Jones *et al*, 2004), Rambach, and Salmonella-Shigella, and Brilliant green agars. These rely on the resistance of salmonellae to a variety of antimicrobials, bile salts, and dyes which inhibit other bacteria, and lack of fermentation of sugars such as lactose and sucrose to differenticiate colonies of *Salmonella* from other bacteria which grow on the media despite the presence of inhibitors (Jones *et al.*, 2004).

None of these media is ideal for all situations; hence the use of two or three media is usually recommended (Doyle and Cliver, 1990). However, the recognition of colonies of *Salmonella* is to a large extent a matter of experience, and their appearance may vary some what, not only from serovar to serovar, but also from batch to batch of the selective culture medium used (ISO 6579, 2002). If *Proteus* species are a problem, the enrichment broths can be incubated at 43°C, or sodium sulphathiazole added to the broths at 0.125mg/100ml. Some laboratories add sodium sulphadiazine to brilliant green agar (80 mg/1) to make it more selective (Quinn *et al*., 1999).

2.6.4. Confirmation

Presumptive *Salmonella* colonies are subcultured and confirmed by means of appropriate biochemical and serological tests. For confirmation, typical or suspect colonies are streaked onto the surface of pre-dried nutrient agar plates, in a manner, which will allow well-isolated colonies to develop. Pure cultures are used for biochemical and serological confirmation (ISO 6579, 2002).

Biochemical testing

Salmonellae are chemoorganotrophic, with an ability to metabolize nutrients by the respiratory and fermentative pathways. The organisms catabolize D-glucose and other carbohydrates with production of acid and gas. Salmonellae are oxidase negative and catalase positive, grow on citrate as the sole carbon source, generally produce hydrogen

sulphide, decarboxylate lysine and ornithine, and do not hydrolyze urea (D'Aoust, 1997; Quinn *et al*., 1999).

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

Salmonellae generally are β-galactosidase, Voges-Proskauer and indole test negative (ISO 6579, 2002). However *Salmonella* species as a biochemically homogeneous group of microorganisms is rapidly diminishing. The situation will likely lead to a reassessment of the diagnostic value of biochemical traits and to their likely replacement with molecular technologies targeted at the identification of stable genetic loci and/or their products that are unique to the genus *Salmonella* (D'Aoust, 1997).

Typing of Salmonella

In epidemiologic investigation and some other situations, it may be necessary to identify the salmonellae to a level of precision beyond the serovar or species (Doyle and Cliver, 1990). Typing method is any method that can be used to differentiate bacteria beyond species level. Outbreak investigations and tracing of zoonotic bacteria among livestock and from livestock via food to man can be performed by the use of bacterial typing methods. These methods can subdivide a bacterial species into individual clonial lines, groups of bacteria produced by the continuous division of cells from the same ancestor (Olsen *et al*., 1993).

There are phenotypic and genotypic typing methods. Originally, phenotypic typing methods, such as serotyping, phage typing and biotyping, are the only methods applied. These traditional methods still play a very important role in tracing of bacteria, and sporadic cases of salmonellosis, for example, will only be grouped to form a tentative outbreak if the isolates show identical serotyping, and where applicable, identical phage

typing results. These methods still provide the basic, definitive background against which more advanced typing methods are applied (Olsen *et al*., 1993).

Serotyping: The final step in the identification of *Salmonella* isolates is serological testing by agglutination assays with antisera specific for somatic (O), flagellar (H), and capsular (Vi) antigens (Doyle and Cliver, 1990; Hirsh, 1999). The O-antigen side chain, polysaccharide portion, is the portion of the lipopolysaccharide (LPS), which is very specific for the different *Salmonella* serovars (Doyle and Cliver, 1990; Coetzer *et al*., 1994; Hirsh, 1999). The kind and number of sugars together with the linkage between them in part determine the antigenic determinants comprising the O-antigens of particular isolate (Doyle and Cliver, 1990).

The other antigen of major interest is the flagellar or H antigen, which is a protienaceous material, and it too is highly specific for species in the genus *Salmonella* (Doyle and Cliver, 1990). Usually the majority of cells have flagella in one phase but there will be a very few cells with the alternative flagellar antigens. The *Salmonella* will agglutinate only with antisera to the flagellar that predominates. To obtain a complete antigenic formula, the phase must be "changed". This involves the selection of the few cells that have the alternative flagellar antigen (Quinn *et al*., 1999). In contrast to somatic antisera, which may produce false positive reactions with closely related nonsalmonellae, the highly specific flagellar antisera provide for the conclusive identification of *Salmonella* strains (D'Aoust, 2000).

The capsular antigen is an acidic polysaccharide, which is expressed only by three species *Salmonella* typhi, *S*. Dublin, and *S.* Hirschfeldii so it is not at all common among the salmonellae. There are also called F antigens or type 1 pili; they are not important in the speciation of *Salmonella* because they are commonly present on the salmonellae and are antigenically similar (Doyle and Cliver, 1990).

The O-antigens together with the antigenic determinants on the surface of the flagella (H-antigens) that are possessed by most salmonellae help serologically to define an isolate. This classification scheme is called Kauffman-White scheme (Hirsh, 1999; Acha and Szyfres, 2001). Based on Kauffman-White scheme approximately 2,500 different

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

Phage typing: Some serovars have several different phenotypes, and their identification can be important in epidemiologic investigation (Acha and Szyfres, 2001). Phage typing schemes for *Salmonella* enterica serovars are based on patterns of lyses produced by distinct phages isolated from a variety of sources (Olsen *et al*., 1993; Quinn *et al*., 1999; Heuzenroeder *et al*., 2004). Pure cultures of bacteria are flooded onto plates and suspensions of typing phages are spotted onto the plates. Strains that are susceptible to infection by the same phages are allocated to the same phage type. As this typing method is cheap and labor inexpensive, it is normally the second method to be applied in the study of *Salmonella* epidemiology, and phage-typing schemes have been developed for many important *Salmonella* serovars. Phage typing is the principal method of typing in *S*. Enteritidis and *S*. Typhimurium (Olsen *et al*., 1993) and has been used to subdivide isolates within serovars Typhi, Typhimurium, Enteritidis, Virchow, Hadar and Heidelberg. Although phage typing is essential for the subdivision of *Salmonella* serovars, the method can prove inadequate for serovars in which a small number of phage types predominate (Heuzenroeder *et al*., 2004).

Antibiogram typing: Resistance to antimicrobial agents is considered to be relatively unstable (Olsen *et al*., 1993), because the majority of bacterial resistance factors are carried on plasmids (genetic determinants) that are often transferable between strains (Olsen *et al*., 1993; Dargatz *et al*., 2003), and which may be dependent on selection pressure to be stably maintained. As an importance, antibiograms are rarely used as the only typing method. The epidemiological significance is secondary to the implications for therapy and control. However, the results of these investigations can readily be used for epidemiological purposes. The spread of multiple antimicrobial resistant strains among livestock is often traced using the antibiogram as a typing method, preferably combined with other typing methods (Olsen *et al*., 1993).

Molecular typing: Genetic typing methods involve plasmid profiling, plasmid restriction analysis, restriction analysis of the full genome and PCR-based typing (Olsen *et al*., 1993).

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

2.7. Antimicrobial resistance of *Salmonella*

Salmonellae are sensitive to a number of antimicrobials, for instance ampicillin, amoxicillin, chloramphenicol, gentamycin, trimethoprim-sulphonamide combinations, fluoroquinolones and nitrofuran derivatives (Seifert, 1996). Early treatment with broad-spectrum antimicrobials and with sulfonamides is highly efficient in preventing deaths and returning animals to normal function (Radostits *et al*., 1994).

The concern with the use of antimicrobials is that they have the potential of predisposing to colonization with salmonellae, increasing level and duration of excretion by carriers and of selecting for antimicrobial resistant strains (Jones *et al*., 2004). However, antimicrobials have been used extensively to treat all animals without undue complications (Radostits *et al*., 1994; House and Smith, 1997; Jones *et al*., 2004). Antimicrobial therapy should be based on results of susceptibility and the information should be used to choose the preferred antimicrobials (House and Smith, 1997; Quinn *et al*., 2002; Jones *et al*., 2004) because R plasmids coding for multiple antimicrobial resistance are comparatively common in salmonellae (Quinn *et al*., 2002).

The effect of antimicrobials on an animal and its resident microorganisms will depend upon the dose level, duration of administration and antimicrobial spectrum of the antimicrobials. The effect of antimicrobials to which salmonellae are sensitive at therapeutic doses must be distinguished from the effects of the same antimicrobial at sub

therapeutic concentrations and the effects at either concentration of antimicrobials to which *Salmonella* are insensitive (Jones *et al*., 2004).

An antimicrobial may have an effect up on the salmonellae or the normal flora or both. Antimicrobial, to which salmonellae are sensitive, used at correct therapeutic dose, may be predicted to reduce excretion. The same antimicrobial at a sub therapeutic dose may lead to increased multiplication of salmonellae due to a suppressive effect on the activity of growth-suppressing normal flora. Similarly, the use of an antimicrobial to which salmonellae are resistant, but the normal flora sensitive, may be expected to result in increased multiplication and excretion of *Salmonella* (Jones *et al*., 2004).

Zoonotic salmonellae usually heal without complications (Acha and Szyfres, 2001). Antimicrobial treatment of uncomplicated salmonellosis is contraindicated because it tends to prolong the carrier state (D'Aoust, 1991). Clinical management of the disease is primarily supportive and rests on fluid and electrolyte replacement (D'Aoust, 1991; Acha and Szyfres, 2001). In uncomplicated *Salmonella* gastroenteritis, patients should be monitored for fluid and electrolyte balance, as in any diarrheal disorder. Antimicrobial therapy does not reduce the duration and severity of symptoms and may in fact prolong convalescence and intestinal carriage of the infecting microorganism. However, some physicians treat infants and elderly persons who have acute gastroenteritis, to prevent complications. Patients with bacteriamia, meningitis, enteric fever, or other extra intestinal infections require antimicrobial treatment (Davis *et al*., 1990).

There is growing concern in the medical and veterinary professions about sources of selection pressure for antimicrobial resistance and cross-species transfer of resistant organisms (Normand *et al*., 2000). Widespread use of antimicrobial agents in food animals is associated with increasing antimicrobial resistance in foodborne pathogens and subsequent multidrug-resistant bacterial infections in human (Angulo *et al*., 2004). In recent years, antimicrobial-resistant *Salmonella* strains have been isolated with increasing frequency (Molla *et al.,* 1999; Gebreyes *et al*., 2000; Mrema *et al*., 2006).

The significance of antimicrobial resistance is most obvious in its impact on treatment of human infections. Prior antimicrobial therapy allows fewer numbers of antimicrobial-resistant salmonellae to cause symptomatic infections; and an increase in the proportion of

salmonellae that are antimicrobial-resistant will increase the overall frequency of salmonellosis (Radostits *et al*., 1994). A study done in Thailand indicated antimicrobial resistance in *Salmonella* isolates from all types of animals and human and higher proportions of multi-drug resistant *Salmonella* isolates from farm workers with livestock contact than among isolates from workers with no livestock contact (Padungtod and Kaneene, 2006). Current attention centers on production animals due to the use of antimicrobials in agriculture and its link with food (Normand *et al*., 2000) which may result in treatment failures, prolonged or more severe illness, increased hospitalization, and increased mortality (Angulo *et al*., 2004). There is also an association between drug-resistant *Salmonella* and the routine clinical use of antimicrobials for infections other than salmonellosis (Radostits *et al*., 1994).

The main cause of antimicrobial resistance in industrialized countries has been the overuse of antimicrobials in animal feeds as a growth enhancer as well as the indiscriminate prescription drug treatment of people and animals. In developing countries, the principal cause of the emergence of multiple antimicrobial resistant *Salmonella* strains may be self-medication, made possible by the public easy access to antimicrobials without prescription (Acha and Szyfres, 2001).

2.8. Prevention and control of salmonellosis

Currently a major effort is being attempted to control *Salmonella* infection in production facilities around the world. In several countries, the application of the hazard analysis critical control point (HACCP) methodology is being used at meat-processing facilities to control pathogen introduction into the food supply (Motsoela *et al*., 2002; Libby *et al.,* 2004).

Hygienic premises, cleanliness, provision of non-contaminated feed and drinking water as well as appropriate feeding are important prerequisites for the prevention of salmonellosis. Infected and/or latently diseased animals have to be separated and treated. They can only return into the herd if they are found to be negative through bacteriological control. In poultry, heat treatment of feed and screening of breeding flocks serologically and destruction of infected ones is practiced (Doyle and Cliver, 1990; Seifert, 1996).

Attempts to control salmonellosis in cattle have involved the use of strict hygienic measure, antimicrobials and vaccination, either singly or in combination. When adult stock has to be bought-in these should be quarantined and examined bacteriologically to prevent the introduction of salmonellosis to herds. Removal of predisposing factors such as dietary imbalance, reduction of stocking densities, segregation of susceptible animals, and prompt antimicrobial treatment of sick animals decreases susceptibility to infection (Jones *et al*., 2004).

Improving the resistance of animals in high-risk groups by immunization is important, as is correct feeding to maintain the intestinal flora in a balanced state. Separation of age groups and the prevention of contact with other infected carrier animals lessen the risk of exposure, while the isolation of diseased animals and the elimination of potential sources of infection prevent the spread of the infection and contamination of the environment (Venter *et al*., 1994).

The growing importance of *Salmonella* as a human disease agent and a major contaminant of the global food chain supports the development of new human and animal vaccines using attenuated *Salmonella* strains that are auxotrophic in the biosynthetic pathways or that lack key virulence determinants. However, the great diversity and rapid succession of serovars in foods and the limited specificity of vaccines for a single or a few closely related serovars pose a major challenge to the development of effective vaccines for meat animals (D'Aoust, 2001).

There are many approaches to control *Salmonella* in foods. The first way is to prevent entrance of the organism through contaminated raw materials. This is particularly important in ingredients, especially in foods of animal origin or foods contaminated by fecal excrement and do not receive heat treatment (Doyle and Cliver, 1990). Important contributing factors in transmission of salmonellae through foods are inadequate cooking; slow cooling of the food, lack of refrigeration for long hours, and inadequate reheating before serving (Acha and Szyfres, 2001). Therefore education of food handlers, both in commercial establishments and in the home, about correct cooking and refrigeration practices for foods of animal origin, and about personal and environmental hygiene are other important control measures to prevent post processing contamination (Doyle and Cliver, 1990; Acha and Szyfres, 2001). However, there is not a definitive skill step of

pathogens in the harvest and processing chain with the exception of cooking; most of the interventions operate on the expectation of a percentage reduction in the numbers of bacteria on the carcass. As such, pre-harvest interventions to lower the load of potential foodborne pathogens in feces of animals presented for harvest are thought to offer opportunities to mitigate the risk of contaminated product. Therefore determining management strategies associated with lower prevalence is a key to decreasing the risk of high pathogen loads at harvest (Dargatz *et al*., 2003).

2.9. Salmonellosis in cattle

In cattle the enteric and septicemic syndromes of salmonellosis are more common than the abortion syndrome. One or more of the syndromes may occur simultaneously in an outbreak of salmonellosis in a herd, or even in a single affected animal. *Salmonella* Dublin is more often the cause of septicemia than *S*. Typhimurium. Calves are more likely than adult animals to suffer from septicemic infections and may develop peracute, acute or chronic salmonellosis. The peracute disease is usually a septicemic condition, it is often fatal, and calves may die suddenly without premonitory signs. Some, however, develop enteritis and diarrhea in addition to septicemia. When the course of septicemia is protracted, signs of hepatitis, pneumonia, meningoencephalitis, polyarthritis and osteomyelitis may develop (Venter *et al*., 1994, Wray and Davies, 2000).

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

Chronic salmonellosis in calves is characterized by unthriftiness, long and scruffy hair coats, and stunting. Diarrhea is not always present and sign of chronic pneumonia with persistent coughing may occur (Wray and Davies, 2000). Calves that survive either

peracute, acute or chronic disease may develop pneumonia, meningoencephalitis, purulent polyarthritis, and osteomyelitis of the vertebrae and bones of the distal parts of the limb, resulting in lameness, paresis or even almost complete paraplegia. Dry gangrene of the skin of the lower limbs and the tips of ears and tail, resembling ergotism, is rarely encountered (Venter *et al*., 1994)

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

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

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

2.10. Public health importance of salmonellosis

Globally, salmonellosis is one of the major foodborne infections in human. The disease situation in human has become increasingly worse during the last decade. According to WHO, the situation has reached alarming proportions in several countries. This is largely a result of the industrialization and large scale intensive production of livestock which has opened the door to the food chain of zoonoses like salmonellosis which cannot be controlled by the traditional post mortem inspection (Lindberg, 1995).

Salmonellosis in human ranges from the generalized typhoid infection, through the less severe paratyphoid infections to a mild gastroenteritis. The majority of serovars produce a mild to severe gastroenteritis that only rarely becomes generalized and severe infections are most often encountered in very young, old or immunologically compromised patients. It is generally accepted that *Salmonella* gastroenteritis is a zoonotic disease, mainly contracted by consuming large numbers of salmonellae in food of animal origin or foods contaminated with animal products in which the salmonellae are proliferated. There is, however, convincing evidence not only that infection can be a sequel to the consumption of small doses but that direct person-to-person contact is involved in many outbreaks (Jones *et al*., 2004).

The clinical condition is generally self-limiting, and remission of the characteristic nonbloody diarrheal stools and abdominal pain usually occurs within 5 days of onset of symptoms. Human infections with nontyphoid strains can also degenerate into systemic infections and precipitate various chronic conditions such as septic reactive arthritis and spondylitis. Preexisting physiological, anatomical and immunological disorders in human hosts can also favor severe and protracted illness through the inability of host defense mechanisms to respond effectively to the presence of invasive salmonellae (D'Aoust, 1997; Lai *et al*., 2005).

2.11. Salmonellosis in Ethiopia

Salmonellosis is considered as one of the most widespread foodborne zoonoses in industrialized as well as in developing countries even though the incidence seems to vary between countries (Acha and Szyfres, 2001). In addition, under-reporting of cases and the presence of other diseases of high priority may overshadow the problem of salmonellosis in some developing countries (D'Aoust, 1994), which includes Ethiopia. Habit of consuming raw and undercooked meat poses high risk of acquiring salmonellosis as well as other zoonotic diseases (Molla *et al.,* 1999; 2003).

In Ethiopia, studies conducted by different individuals on slaughtered swine, avian, bovine, camel, ovine and caprine species indicated the presence of a number of *Salmonella* serovars (Table 1) indicating the potential of carcass contamination during slaughtering operations.

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

Species	Sample	No. of animals		Serovars isolated	Reference
		Examined	Positive		
Bovine	MLN*	280	6	*S*. Dublin, *S*. Bredeny	Pegram *et al*. (1981)
Porcine	MLN (pooled)	160	1	*S*. Saintpaul	"
Avian	Organs	-	77	*S*. Gallinarum	"
Bovine	feces (pooled)	235	5	*S*. Dublin, *S*. Muenchen	Nyeleti *et al*. (2000)
"	MLN (pooled)	235	9	*S*. Anatum, *S*. Dublin	"
"	AM	235	23	*S*. Anatum, *S*. Dublin	"
"	DM	235	28	S. Anatum, S. Dublin	"
Bovine	feces (pooled)	323	2	*S*. Mishmarhaemek	Alemayehu *et al*. (2003)
"	MLN	323	3	*S*. Typhimurium, *S*. Enteritidis	"
"	AM	323	9	*S*. Mishmarhaemek , *S*. Dublin, *S*. Typhimurium , *S*. Guildford	"
"	DM	323	10	*S*. Dublin, *S*. Mishmarhaemek , *S*. Typhimurium , *S*. Guildford, *S*. Enteritidis	"
Camel	feces	119	18	*S*. Saintpaul, *S*. Muenchen, *S*. Kottbus, *S*. Havana, *S*. Heidelberg, *S*. Derby, *S*. Enteritidis, *S*. Anatum	Molla *et al*. (2004)
"	MLN	119	19	*S*. Saintpaul, *S*. Braenderup, *S*. Muenchen, *S*. Typhimurium var. Copenhagen, *S*. Kottbus, *S*. Hadar, *S*.Bovismorbificanse, *S*. Butantan, *S*. Infantis	"
"	liver	119	14	*S*. Saintpaul, *S*. Braenderup, *S*. Typhimurium var. *S*. Hadar, Copenhagen, *S*. Kottbu	"

Species	Sample	No. of animals		Serovars isolated	Reference
		Examined	Positive		
"	spleen	119	17	*S*. Saintpaul, *S*. Typhimurium var. Copenhagen, *S*. Heidelberg, *S*. Braenderup, *S*. Infantis, *S*. Kottbus, *S*. Anatum, *S*. Butantan, *S*. Havana	"
"	AM	119	25	*S*. Saintpaul, *S*. Typhimurium, *S*.Braenderup, *S*. Muenchen, *S*. Kottbus, *S*. Hadar, *S*. Havana	"
"	DM	119	23	*S*. Saintpaul, *S*. Braenderup, *S*. Havana, *S*. Infantis, *S*. Muenchen, *S*. Kottbus	"
Ovine	Feces	104	5	*S*. Typhimurium, *S*. Enteritidis, *S*. Reading, *S*. Heidelberg	Molla *et al.* (2006a)
"	MLN	104	8	*S*. Typhimurium var. Copenhagen, *S*. Typhimurium, *S*. Reading, *S*. Give, *S*. Heidelberg, *S*. Niederoderwitz	"
"	Liver	104	2	*S*. Typhimurium, *S*. Give	"
"	AM	104	2	*S*. Typhimurium, *S*. Heidelberg	"
"	Spleen	104	1	*S*. Typhimurium,	"
Caprine	Feces	100	2	*S*. Typhimurium, *S*. Poona	
"	MLN	100	2	*S*. Poona	"
Ovine	Feces	47	1	*S*. Kottbus	Woldemariam *et al*. (2005)
"	Liver	47	2	*S*. Infantis	"
"	AM	47	5	*S*. Infantis, *S*. Braenderup	"
"	DM	47	2	*S*. Kingbawa, *S*. Infantis	"

Species	Sample	No. of animals		Serovars isolated	Reference
		Examined	Positive		
Caprine	Feces	60	2	*S*. Infantis, *S*. Kottbus	"
"	MLN	60	7	*S*. Zanzibar, *S*. Infantis, *S*. Anatum, *S*. Butantan, S. Typhimurium, *S*. Kingbawa	"
"	Spleen	60	2	*S.* Infantis, *S.* Butantan	"
"	Liver	60	3	*S*. Butantan, *S*. Braenderup	"
"	AM	60	2	*S.* Butantan, *S.* Infantis	"
"	DM	60	7	*S.* Hadar, *S*. Infantis, *S.* Butantan	"
Bovine	HS	100	31	*S*. Anatum, *S.* Newport*, S*. Bredeney, *S.* Eastbourne,	Sibhat (2006)
"	RC	100	19	*S*. Uganda, *S*. Anatum *S.* Newport	"
"	CC	100	6	*S.* Typhymurium, *S*. Anatum *S.* Newport	"
"	MLN	100	8	*S*. Reading, *S*. Anatum *S.* Newport	"
"					
"	CS	100	2	*S.* Eastbourne, *S*.Urbana	"
Porcine	MLN	101	42	*S.* Hadar, *S*. Kentuckey, *S*. Anatum *S*. Havana, *S*. Leoben, *S*. Enteritidis, *S*. Blockley, *S.* Kiambu *S.* Livingstone	Molla *et al*. (2006b)
"	Tongue	101	22	*S.* Hadar, *S.* Kentuckey, *S*. *A*natum, *S*. Blockley, *S*. Leoben, *S*. Havana, *S.* Gaminara, *S*. Eastbourne	"
"	CC	101	17	*S.* Hadar, *S*. Kentuckey, *S*. Blockley, *S.* Leoben, *S.* Gaminara, *S*. Anatum	"
"	Liver	99	11	*S.* Hadar, *S*. Uganda, *S*. Anatum, *S*. Kentuckey, *S*. Havana, *S.* Blockley,	"

Species	Sample	No. of animals		Serovars isolated	Reference
		Examined	Positive		
"	Muscle	99	2	*S.* Newport, S. Kentuckey	"
"	MLN	278	99	*S.* Typhimurium var. Copenhagen, *S.* Eastbourne, *S.* Saintpaul *S.* Newport, *S.* Kentuckey	Aragaw *et al*. (2007)
"	CC	278	63	*S.* Anatum *S.* Kentuckey, *S.* Hadar *S.* Typhimurium var. Copenhagen *S.* Saintpaul, *S.* Eastbourne	"
"	Carcass	278	11	*S.* Hadar, *S.* Eastbourne, *S.* Havana, *S.* Anatum, *S.* Kentuckey	"

* LMN = Mesenteric lymph nodes, AM = Abdominal muscles, DM = Diaphragmatic muscle, HS = hide swab, RC = Rumen content, CC = Caecal content, CS = Carcass swab.

Food, containing products from farm animals, especially from poultry, pigs, and cattle are an important source of human *Salmonella* infections (van Duijkeren *et al*., 2002). Ejeta *et al*. (2004) reported that *Salmonella* is widespread in minced beef, mutton, and pork samples obtained from retail supermarkets in Addis Ababa, Ethiopia. Various serovars of *Salmonella* were also isolated from samples of meat (Tibaijuka *et al*., 2003; Molla and Mesfin, 2003; Zewdu 2004), ready to eat foods (Zewdu, 2004; Tegegne and Ashenafi, 1998) and other food items in Ethiopia (Zewdu, 2004) (Table 2) indicating the potential of human infections through consumption of raw or undercooked food of animal origin.

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

Sample type	No. of samples		Serovars isolated	Reference
	Examined	Positive		
Ayib	84	1	S. Braenderup	Abdella *et al*. (1996)
Raw milk	159	1	S. Hall	"
Kitfo	73	1	S. Hall	"
Raw 'Kitfo'	50	21	-	Tegegne and Ashenafi (1998)
Minced beef	330	26	*S*. Anatum, *S*. Dublin, *S*. Saintpaul	Nyeleti *et al*. (2000)
Chicken meat and giblets	301	54	*S*. Braenderup, *S*. Anatum, *S*. Saintpaul, *S*. Uganda	Tibaijuka *et al*. (2003)
Chicken meat and giblets	378	80	*S*. Braenderup, *S*. Typhimurium var. Copenhagen, *S*. Anatum, *S*. Kottbus and *S*. Typhimurium	Molla and Mesfin (2003)
Minced beef	160	23	*S*. Infantis, *S*. Braenderup, *S*. Anatum, *S*. Bovismorbificans, *S*. Vejle, *S*. Dublin, *S*. Saintpaul	Ejeta *et al.* (2004)
Mutton	85	12	*S*. Infantis, *S*. Braenderup, *S*. Anatum, *S*. Bovismorbificans	"
Pork	55	9	*S*. Infantis, *S*. Braenderup, *S*. Vejle	"
Chicken meat	208	29	*S*. Newport, *S*. Braenderup, *S*. Hadar, *S*. Typhimurium, *S*. Kentucky, *S*. Anatum	Zewdu (2004)
Pork	194	22	*S*. Newport, *S*. Infantis, *S*. Kottbus	"
Mutton	212	23	*S*. Newport, *S*. Hadar, *S*. Typhimurium, *S*. Kentucky, *S*. Anatum	"
Minced beef	142	12	*S*. Newport, *S*. Typhimurium, *S*. Infantis, *S*. Kentucky, *S*. Anatum, *S*. Saintpaul	"
Fish	128	3	*S*. Newport	"
Cheese	190	4	*S*. Newport	"

Surveillance of *Salmonella* serovars from human and animal sources is relevant for detecting outbreaks, for identifying sources of infection and for implementing prevention

and control measures (van Duijkeren *et al*., 2002). Nontyphoidal salmonellae are reported as important causes of diarrhea in Ethiopia (Mache, 2002; Ashenafi and Gedebou, 1985; Mache *et al*., 1997). Sibhat (2006) isolated *S.* Anatum *S.* Newport from flayers and eviscerators hands swab and Nyeleti et al. (2000) from stool of slaughterhouse personnel (Table 3), indicating possibility of carcass contamination by Salmonella carrier individuals during the slaughtering operation.

Table 3: *Salmonella* serovars/serogroups isolated from human in Ethiopia

Sample type	No. of samples		Serovars/serogroups isolated	Reference
	Examined	Positive		
Blood, stool			Serogroup A, B, C, E, S. Typhi	Gedebou and Tassew (1981)
Stool	1000	45	Serogroup A, C, B, D, E, S. Typhi	Ashenafi and Gedebou (1985)
Stool	700	45	Serogroup A, B, C, D, E, S. Typhi	Mache et al. (1997)
Stool	300	18	S. Anatum, S. Dublin, S. Meleagridis	Nyeleti et al. (2000)*
Stool	384	59	Serogroups A, B, C, D, E, S. Typhi	Mache (2002)
Stool	68	5	S. Newport	Zewdu (2004)**
FHS*	100	7	*S.* Anatum *S.* Newport	Sibhat (2006)**
EHS	100	2	*S*. Anatum *S.* Newport	"

*FHS = Flayers hand swab, EHS = Eviscerators' hand swab, ** non-clinical samples

3. MATERIALS AND METHODS

3.1. Study area

Bahir Dar is located at 490 kms northwest from Addis Ababa, Ethiopia. Bahir Dar has an altitude of 1700m above sea level, average annual temperature of 28^{o}C, and annual rainfall of 1600 mm (NMSA, 1999). Bahir Dar municipality abattoir, the study area, is located in the southern border of the town. The abattoir has 17 abattoir workers, one meat inspector, and two guards. The meat inspector is at the level of animal health assistant. Most of the abattoir workers are, however, untrained personnel.

In the campus of the slaughterhouse there are two offices for reception and recording of cases. There is a separate building for managing and storing of skin. The slaughterhouse has four rooms, two rooms for Muslims and the other two for Christians use. The lairage has a capacity for keeping 60 animals at a time. Forty to 55 heads of cattle are slaughtered each day except Tuesdays and Thursdays, in non-fasting seasons and 10 to 20 animals during the period of fasting. Only adult cattle were slaughtered during the study period at the abattoir.

3.2. Study animals

The study was conducted on 186 apparently healthy slaughtered cattle at Bahir Dar municipality abattoir from October 2006 to March 2007. The animals, which were slaughtered at Bahir Dar municipality abattoir, originated mostly from west Gojam, and South Gondar Zones of Amhara National Regional State. Animals transported from Adet, and Wereta markets and those including animals from Bahir Dar were kept for 24 hrs to fifteen days at Bahir Dar before being presented to the abattoir for slaughter.

During their stay in their respective owners, animals were kept in open-air during the day and in barns during the night and fed hay and water until they are presented for slaughter. Animals could also be bought and presented to the abattoir on the same day for slaughter. After they were presented to the abattoir they were kept for an average of 7 hrs without

feed and water in holding pens. All slaughtered animals were adult male cattle and the majority of them were those which have finished their traction life.

3.3. Study design

The study was a cross-sectional study involving 186 male cattle slaughtered between October 2006 and March 2007 at Bahir Dar municipality abattoir. The variable of interest considered as an output variable versus risk factors at the abattoir was carcass bacteriological status. The explanatory variables considered at the abattoir were animal intestinal content, mesenteric lymph nodes and liver tissue bacteriological status.

3.3.1. Sample size

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

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

Where:

n = required sample size

Pexp = expected prevalence

d = desired absolute precision

A previous study on *Salmonella* in commercial slaughterhouses in Debre Zeit, Ethiopia revealed prevalence of 14% (Sibhat, 2006). Debre Zeit has a similar agro-ecological situation with that of Bahir Dar; therefore, using 14% expected prevalence and 5% absolute precision at 95% confidence level, the number of animals needed to estimate the prevalence of *Salmonella* in Bahir Dar municipality abattoir was calculated to be186.

3.3.2. Sampling procedure

Samples were collected every Friday of the week or every Friday of the other week. Animals were selected with simple random sampling using the numbers given both for antemortem and postmortem inspection.

On each sampling day, animals were selected randomly using the identification numbers given to the animals both for antemortem and postmortem examination. After selecting and recording the identification of the selected animals liver tissue, mesenteric lymph nodes, intestinal content and carcass swab were aseptically collected in sterile containers. Liver tissue (more than 25 gm) was collected from each of the selected animals after evisceration. The mesentery with the lymph nodes attached was removed from the surrounding structures and brought to the laboratory in separate sterile plastic bags. Intestine containing approximately 25 gm of its content was collected in sterile universal bottles by cutting at both ends with scalpel blade. Separate sterile scalpel blades and sterile forceps were used for each and every cutting during sample collection.

Swab samples were collected aseptically from each of selected animal carcasses. Sterile cotton swabs moistened in 10 ml buffered peptone water (BPW) (AES loboratoire, Cedex, France) were rubbed on both sides of the carcass from hindquarter to the forequarters uniformly. Swabbing of carcass was conducted holding the sterile sticks on opposite end of its tip; cotton rolled over it, with hands in sterile gloves. The swab sample was inserted into the universal bottles containing buffered peptone water after cutting off the part of the stick, which was in contact with the hand, by bending out on the mouth of the bottle. Carcass swab samples were collected at the end of slaughtering process before it is prepared for loading.

At the end of each sample collection, every sampling bottle was labeled including date of sampling and the type of sample collected corresponding to the animal identification number. The samples were then transported in cooler boxes with ice to Bahir Dar Regional Veterinary Laboratory and stored in a refrigerator at 4°C for not more than 12 hrs before they were being processed.

3.3.3. Sample processing

In the laboratory the lymph nodes were aseptically freed from surrounding tissues and 25 gram was weighed in sterile aluminum foil. All outer surface of the weighed tissue of the lymph nodes passed over flame to avoid contamination and thoroughly minced into very small, fine pieces, on sterile plate using sterile scalpel blade. The minced lymph nodes were put into sterile stomacher bags and 225 ml BPW was added and homogenized manually by shaking for 3-4 minutes. Twenty-five grams of liver was weighed, minced and added into 225 ml of BPW and was agitated as that of lymph node samples.

Twenty-five grams of the intestinal content was weighed on sterile aluminum foil and put in sterile stomacher bags. About 225 ml BPW was added and agitated manually to disperse the content. Each carcass swab was agitated manually while in their original sterile universal bottle containing 10 ml BPW.

Whenever samples were found less than 25 gm, BPW was added to the samples in ratio of 1:10 (ISO 6579, 2002).

3.4. Isolation and identification of *Salmonella*

Recent emphasis on food safety issues and the development of guide lines using *Salmonella* as an indicator for enteric contamination of meat and potential pathogen in animal products has led to efforts to standardize detection techniques (Maddox, 2003). International organization for standardization (ISO) specifies food and animal feeding stuffs-horizontal method for the detection of *Salmonella* species should be based on the standard detection of *Salmonella* through different successive stages as shown in figure 1 (ISO 6579, 2002). Therefore the isolation and identification was made based on this standard. Quinn *et al*. (1999) had also been used to complement media preparation and in identifying colonies. The bacteriological media used in different stages were prepared according to the manufacturer's recommendations (Appendix I).

3.4.1. Pre-enrichment in non-selective liquid medium

Appropriate amount of processed samples (about 25 gm) were pre-enriched in 225 ml of buffered peptone water (BPW). This step in the detection of *Salmonella* involves inoculation of BPW with the test portion, and incubation at 37°C ± 1°C for 16 h to 20 h (ISO 6579, 2002). Mesenteric lymph nodes, liver tissue samples, and intestinal content were incubated in the stomacher bags whereas swab samples were incubated while they are in the universal bottles.

3.4.2. Enrichment in selective liquid media

Enrichment in selective liquid media was done by transferring 0.1ml of the culture obtained from the non-selective pre-enrichment medium to a tube containing 10 ml of a Rappaport-Vassiliadis with Soya broth (RVS broth) and 1ml to a tube containing 10 ml of Muller-Kauffmann tetrathionate novobiocin broth (MKTTn broth) (Oxoid, Hampshire, England). The inoculated RVS broth (Titan Biotech, Raj, India) medium was incubated at 42°C for 24 h ± 3 h, whilst Muller-Kauffmann tetrathionate novobiocin broth was incubated at 37°C ± 1°C for 24 h ± 3 h (ISO 6579, 2002).

3.4.3. Selective plating and identification

After incubation for 24 h ± 3 h, the cultures obtained in the RVS broth and MKTTn broth, a loopful of inoculum from each of enrichment cultures were inoculated on the surface of two different Petri dishes containing xylose lysine deoxycholate (XLD) agar (AES laboratoire, Cedex, France), the first selective plating-out medium. The inoculated plates were incubated at 37 ^{o}C for 24 h ± 3 h. In the standard protocols of ISO procedure, plating-out and identification of *Salmonella* involves inoculation of two selective solid media and the choice of the second medium is left to the discretion of the testing laboratory unless there is a specific international standard relating to the product to be examined. However the manufacturer's instructions should be followed regarding its preparation for use (ISO 6579, 2002). Therefore we selected MacConkey agar (Titan Biotech Limited, Bhiwadi,

India) based on its availability as the second plating-out medium and it was prepared strictly based on the instruction of the producer.

A loopful of the inoculum from each of the enrichment cultures were inoculated on to the surface of each of two different plates containing the second selective plating-out medium (MacConkey agar) as that of the first plating-out medium. The inoculated plates were incubated at 37°C for 24 hours (Quinn *et al*., 2002).

The plates were examined for the presence of presumptive *Salmonella* colonies after the end of incubation. *Salmonella* will first ferment xylose creating a temporary acid reaction and appears yellow but this is reversed by the subsequent decarboxylation of lysine with alkaline metabolic products (Quinn *et al.,* 1999; ISO 6579, 2002). Typical *Salmonella* colonies grown on XLD agar have a black center due to production of hydrogen sulphide (H_2S) and a lightly transparent zone of reddish color due to the color change of the indicator (ISO 6579, 2002). Lactose-positive *Salmonella* grown on XLD agar become yellow with or without blackening (Quinn *et al.,* 1999; ISO 6579, 2002). *Salmonella* unable to utilize lactose in MacConkey but attacks the peptone (nitrogen source) in the medium with resulting alkaline metabolic products so both the medium and colonies are pale straw-colored (Quinn *et al.,* 1999; Quinn *et al.,* 2002).

3.4.4. Confirmation

For confirmation, at least five presumptive (typical or suspect) *Salmonella* colonies were selected from each plating-out medium. If there were fewer than five typical or suspect colonies on one plate, all the typical or suspect colonies were taken and streaked onto the surface of pre-dried nutrient agar (Oxoid, Hampshire, England) plates in a manner, which allow well isolated colonies to develop. The inoculated plates were incubated at 37°C for 24 h ± 3 hours. Pure cultures obtained on nutrient agar were used for biochemical and serological confirmation (ISO 6579, 2002).

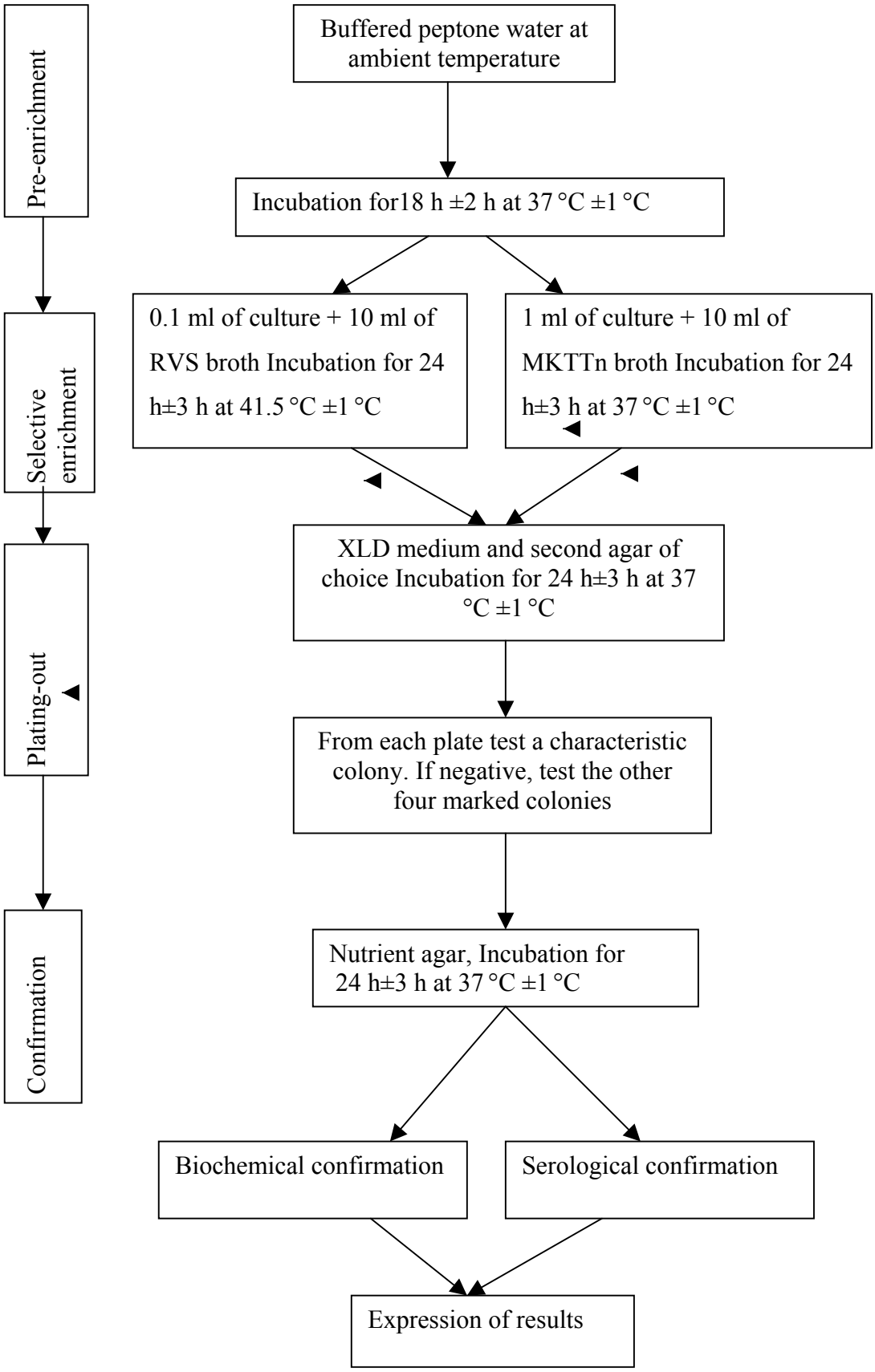

Figure 1: Horizontal method for the detection of *Salmonella* (ISO 6579, 2002)

Biochemical tests: Pure cultures obtained from nutrient agar were used for biochemical confirmation. Triple sugar iron agar (TSI) (Difco, Becton Dickinson, Claix, France) slants were inoculated from pure cultures by streaking the slant and stabbing the butt and without flaming the wire lysine iron agar (LIA) (Difco™, Becton Dickinson, Claix, France) was inoculated just below the surface and both tubes were incubated at 37°C ± 1°C for 24 h ± 3 h loosely capped to maintain aerobic conditions and to prevent excessive H_2S production. Pure isolates were inoculated on urea agar (BBL®, Becton Dickinson, USA) and Simons's citrate agar (Difco, Detroit, USA) by streaking the slants. Both of the inoculated tubes were incubated at 37°C ± 1°C for 24 h ± 3 h (ISO 6579, 2002) and changes in the inoculated media were interpreted for *Salmonella* after the end of incubation following the guide line.

Typical *Salmonella* cultures in TSI agar show alkaline (red) slants, and acid (yellow) butts with gas (bubbles) formation and (in about 90% of the cases) formation of hydrogen sulfide (H_2S) (blackening of the agar). Alkaline reaction (purple color) both in the slant and the butt superimposed with H_2S after incubation indicates a typical positive reaction for *Salmonella* in lysine iron agar. Positive reaction in urea changes the color of phenol red to rose pink and latter to deep cerise due to liberation of ammonia as the result of splitting of urea. However, typical *Salmonella* colonies do not hydrolyze urea; therefore the medium remains yellow (negative test) (ISO 6579, 2002). *Salmonella* colonies grown on Simons's citrate agar produce alkaline products using the medium as a sole carbon source hence deep blue color indicates positive reaction.

Isolates presumptive of *Salmonella* on the biochemical tests were cultured on brain heart infusion agar (Difco, Becton Dickinson, Claix, France). One isolate from each positive sample was sent to Agence Française de Securite Sanitaire des Aliments (AFSSA), Unité, Caractérisation et Epidémiologie Bactérienne, Maisons-Alfort, Cedex, France for serotyping.

Salmonella serotyping: Serotyping of the *Salmonella* isolates were carried out at the Agence Française de Securite Sanitaire des Aliments (AFSSA), Unité, Caractérisation et Epidémiologie Bactérienne, Maisons-Alfort, Cedex, France. For serotyping, the somatic (O) antigens of the *Salmonella* isolates were determined with slide agglutination test as

described by Ewing (1986), whereas the flagellar (H) antigens were identified by using a microtechnique (Shipp and Rowe, 1980) that employs microtitre plates. The antigenic formulae of *Salmonella* serovars listed by Popoff and Le Minor (1997) were used to name the serovars.

3.5. Antimicrobial resistance test

Isolates biochemically and serologically confirmed as *Salmonella* were tested for their resistance to individual antimicrobial drug by the disk diffusion technique (NCCLS, 1997). Four to five well-isolated colonies grown on nutrient agar were transferred on to tubes containing 5 ml of tryptic soy broth (Oxoid, England). The broth culture was incubated at 35°C for 4 hrs until it achieves or exceeds the 0.5 McFarland turbidity standard (Appendix II). For those tubes, which exceeded the turbidity standard, adjustments were made by adding sterile saline solution to obtain turbidity visually comparable to the standard.

Within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile swab was immersed in each of the dilution suspension and swabbed uniformly over the surface of two plates of Muller Hinton agar (Difco, Becton Dickinson, Claix, France) for each inoculum. The plates were held at room temperature for 30 minutes to allow drying. Using sterile forceps, disks impregnated with known concentration of antimicrobials were dispensed onto the surface of Muller Hinton agar plates. The plates were incubated at 37°C for 20 hrs and examined for zones of inhibition. The diameters of the zones of inhibition were recorded to the nearest millimeter, and classified as resistant, intermediate, or susceptible according to published interpretive chart (NCCLS, 1997). The type of tested antimicrobials, their concentration in the discs and their zone of inhibition in deciding susceptibility are given in table 4.

Table 4: Antimicrobials and their concentrations used to test susceptibility of isolates

Antimicrobial agent	Symbol	Amount/disk	Zone diameter (mm)		
			Resistant	Intermediate	Susceptible
Ampicillin	Amp	10 μg	≤ 13	14-16	≥17
Chloramphenicol	Chl	30 μg	≤ 12	13-17	≥ 18
Gentamycin	Gen	10 μg	≤ 12	13-14	≥ 15
Norfloxacin	Nor	10 μg	≤12	13-16	≥17
Polymyxin-B	Pol	300 IU	≤ 8	9-11	≥12
Streptomycin	Str	10 μg	≤ 11	12-14	≥ 15
Tetracycline	Tet	30 μg	≤ 14	15-18	≥ 19
Trimethoprim	Tri	5 μg	≤10	11-15	≥16

3.6. Data management and analysis

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

The significance of differences between the prevalence of *Salmonella* species in various sample types was determined using Fisher's exact test when the numbers within categories were too small for the Chi-square test. The explanatory variables (liver tissue, mesenteric lymph nodes, and intestinal content *Salmonella* status) were considered as risk factors and separately analyzed using logistic regression analyses to see their association with the outcome of the bacteriological status of the carcass.

4. RESULTS

The present study was conducted on 186 apparently healthy slaughtered cattle at Bahir Dar municipality abattoir from October 2006 to March 2007 to estimate the prevalence and distribution of *Salmonella* and to find out the diversity and the antimicrobial resistance patterns of *Salmonella* serovars. Bacteriological examination was conducted on liver tissue, mesenteric lymph nodes (MLN), intestinal content (IC), and carcass swab (CS) samples (each n = 186).

4.1. Prevalence of *Salmonella*

In studying the prevalence at animal level, an animal was considered *Salmonella* positive when it was bacteriologically positive either for one, two or all of liver tissue, mesenteric lymph nodes and intestinal content samples. The status of *Salmonella* on the carcass has been considered as indicator of contamination. However, a sample was considered *Salmonella* positive when it was bacteriologically positive for *Salmonella* in determining sample prevalence.

Out of the total 186 cattle examined 7% (13 of 186) were bacteriologically positive for *Salmonella.* Different level of detection of *Salmonella* was observed in different sample types of *Salmonella* positive animals. *Salmonella* was detected only from their intestinal content in 53.8% (7 of 13) of culture positive animals. In three animals, *Salmonella* was isolated both from their mesenteric lymph nodes and their intestinal content. However *Salmonella* was isolated neither from liver alone nor from liver and intestinal content together.

Of the total 744 samples taken from 186 animals, 3.8% (28 of 744) were culture positive for *Salmonella*. Salmonellae were detected from all sample types and there was considerable variation in frequency of isolation among the different sample types (Table 5). However, there was no significant difference between the prevalence in the different samples (Fisher's exact = 0.06, $P > 0.05$).

Table 5: Prevalence of *Salmonella* isolates in different sample types

Sample type	Number of samples		95% Confidence interval
	Examined	Positive (%)	
Liver	186	2 (1.1)	0.4 - 2.5
Mesenteric lymph node	186	6 (3.2)	0.7 - 5.8
Intestinal content	186	11 (5.9)	2.5 - 9.3
Carcass swab	186	9 (4.8)	1.7 - 7.9
Total	744	28 (3.8)	2.4 - 5.2

Fisher's exact = 0.06, P > 0.05

The level of carcass contamination was considered as dependent variable taking liver, mesenteric lymph nodes, and intestinal content *Salmonella* status as a risk factor for carcass contamination. Therefore association of carcass contamination with liver, mesenteric lymph nodes, and intestinal content *Salmonella* status was assessed using logistic regression analysis but no association was found between carcass contamination and any of the other variables.

The highest sample prevalence 5.9% (11 of 186) was found on intestinal content, which contributed 39.3% (11 of 28) of the total isolates while the lowest prevalence 1.1% (2 of 186) was found on liver tissue samples contributed 7.1% (2 out of 28) of the total isolates. The proportion of *Salmonella* in liver tissue, mesenteric lymph nodes, intestinal content, and carcass swab samples from total isolates is presented in figure 2.

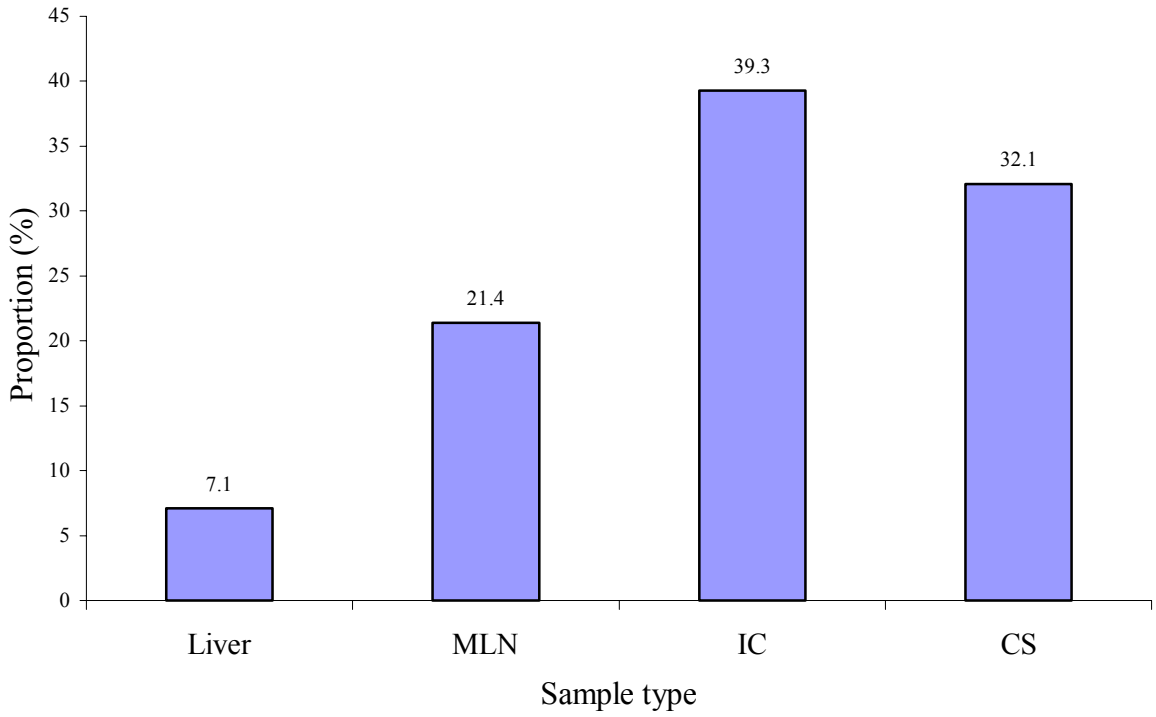

Figure 2: Proportion of positive samples from total isolates

4.2. Distribution of *Salmonella* serovars

Excluding four untypable (rough strains), the 28 isolates consist of six different *Salmonella* serovars. Isolated serovars include *Salmonella* Typhimurium, *S.* Newport, *S.* Haifa, *S.* Heidelberg, *S.* Infantis and *S.* Mishmarhaemek. *Salmonella* Typhimurium and *S.* Newport were the two most frequently isolated serovars each accounted 21.4% (6 of 28) of the total isolates (Table 6).

Table 6: *Salmonella* serovars isolated from different sample types

Serovars	Liver	MLN	IC	CS	Total (%)
S. Typhimurium	1	2	1	2	6 (21.4)
S. Newport	1	1	3	1	6 (21.4)
S. Haifa		1	1	1	3 (10.7)
S. Heidelberg			1	1	2 (7.1)
S. Infantis		1	2	2	5 (17.9)
S. Mishmarhaemek			1	1	2 (7.1)
Untypable serovars		1	2	1	4 (14.3)
Total	2	6	11	9	28 (100)

Salmonella Heidelberg and *S.* Mishmarhaemek were least frequently isolated. Each of them was detected in frequency of 7.1% (2 of 28) of the total isolates. Four of the total isolates were untypable. The prevalence of *Salmonella* serovars in positive samples is presented in figure 3.

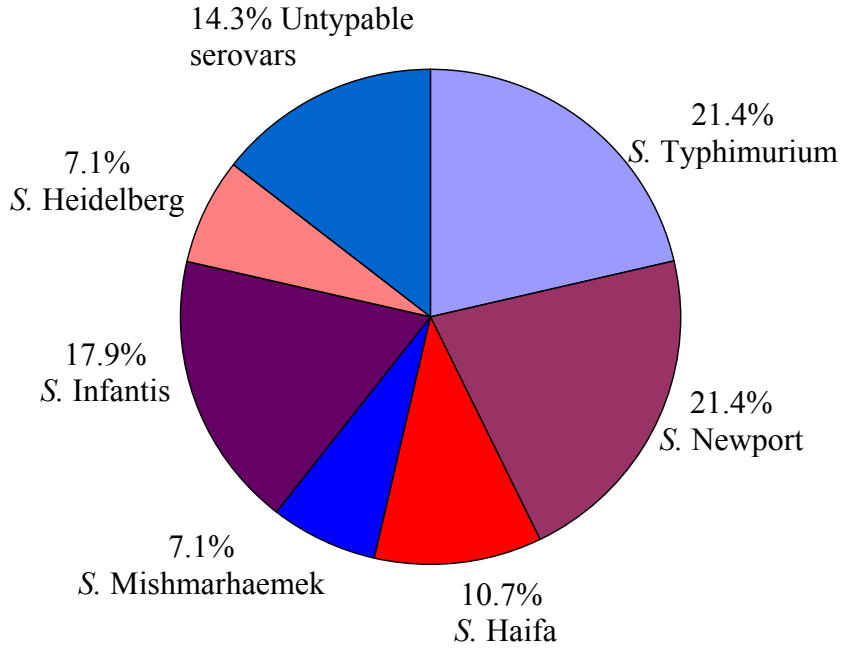

Figure 3: Prevalence of *Salmonella* serovars expressed as percentage of the total isolates

Even though the isolates were of same serovars in 10 of the animals, more than one isolate was detected in 11 of the animals which were positive for *Salmonella* (Table 7). All the six

serovars were detected both from mesenteric lymph node, intestinal content, and carcass swab samples except two serovars (*S.* Mishmarhaemek and *S.* Heidelberg), which were not detected from samples of mesenteric lymph nodes.

Table 7: Distribution of serovars on samples of *Salmonella* positive animals

Animal number	Sampling date	Status of *Salmonella*			
		Liver	MLN	IC	CS
19	12/01/07	-	-	*S.* Newport	Untypable
23	19/01/07	-	*S.* Infantis	*S.* Newport	*S.* Infantis
27	19/01/07	-	*S.* Typhimurium	-	*S.* Typhimurium
42	26/01/07	-	-	*S.* Infantis	*S.* Infantis
54	02/02/07	-	-	*S.* Typhimurium	*S.* Typhimurium
74	02/02/07	*S.* Typhimurium	*S.* Typhimurium	*S.* Newport	-
91	09/02/07	-	-	*S.* Haifa	*S.* Haifa
97	16/02/07	-	-	Untypable	*S.* Newport
128	23/02/07	*S.* Newport	*S.* Newport	-	-
135	02/03/07	-	Untypable	*S.* Heidelberg	*S.* Heidelberg
151	09/03/07	-	*S.* Haifa	*S.* Mishmarhaemek	-
167	09/03/07	-	-	Untypable	*S.* Mishmarhaemek
178	16/03/07	-	-	*S.* Infantis	-

4.3. Antimicrobial resistance patterns

Antimicrobial sensitivity of the 28 isolates was tested for eight different antimicrobials based on the availability of drugs. Eleven of the total 28 isolates (39.3%) were resistant to one or more of the tested antimicrobials. Of these resistant isolates 14.3% (4 of 28) were multiple antimicrobial resistant while 25% (7 of 28) were resistant to single antimicrobial (Table 8). The highest number of resistant isolates was detected from intestinal content. Of the total isolates detected from intestinal content, four isolates were resistant to a single antimicrobial while one was multiple antimicrobial resistant (resistant to more than one antimicrobial).

Table 8: Antimicrobial resistant *Salmonella* isolates by the type of samples

Sample type	Isolates	Resistant isolates to		
		Single resistant (%)	MDR*	Total (%)
Liver	2	-	-	-
Mesenteric lymph nodes	6	1(16.7)	3 (50)	4 (66.7)
Intestinal content	11	4 (36.4)	1 (9.1)	5 (45.5)
Carcass swab	9	2 (22.2)	-	2 (22.2)
Total	28	7 (25)	4 (14.3)	11 (39.3)

MDR = Multiple antimicrobial resistant

Both single and multiple antimicrobial resistant isolates of intestinal content contributed 45.5% (5 of 11) of the total resistant isolates. When the number of single and multiple antimicrobial resistant isolates was compared, single resistant isolates were higher in intestinal content than mesenteric lymph nodes (Figure 4).

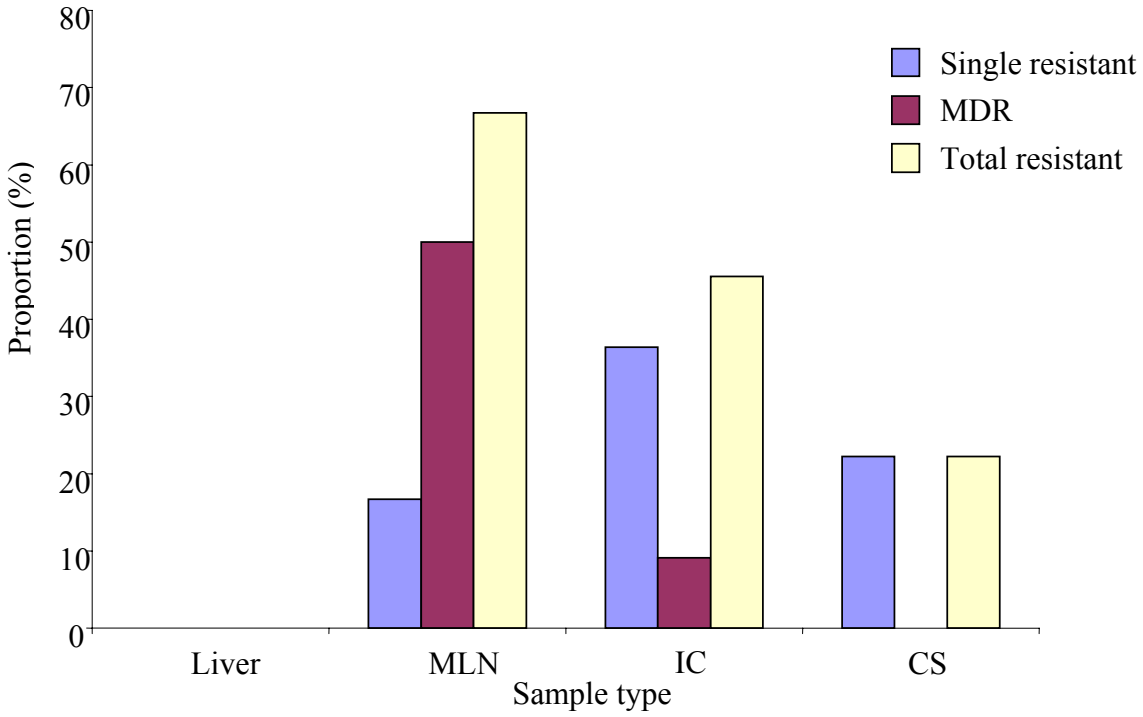

Figure 4: Proportion of antimicrobial resistant isolates by sample type

All isolates were susceptible to the antimicrobial effects of gentamycin, norfloxacin, and trimethoprim. From 224 drug-isolates combination it was observed that largest number of isolates 150 of 224 (67%) were susceptible. Seven (28%) to 15 (53.6%) isolates were intermediate resistant five of the antimicrobials.

Eleven of the 28 isolates (39.3%) were resistant to streptomycin followed by 14.3% (4 of 28) resistant isolates to tetracycline. Resistant isolates to ampicillin were 7.1% (2 of 28) of the total isolates which were relatively less in number when compared to isolates resistant to streptomycin. There was only one isolate resistant to chloramphenicol (Table 10).

Table 9: Antimicrobial susceptibility of isolates to each of the antimicrobials

Type of antimicrobial	Number of isolates			Total
	Resistant (%)	Intermediate (%)	Susceptible (%)	
Chloramphenicol	1 (3.6)	7 (25)	20 (71.4)	28
Ampicillin	2 (7.1)	11(39.3)	15 (53.6)	28
Gentamycin	-	-	28 (100)	28
Norfloxacin	-	-	28 (100)	28
Polymyxin B	-	15 (53.6)	13 (46.4)	28
Streptomycin	11 (39.3)	8 (28.6)	9 (32.1)	28
Tetracycline	4 (14.3)	15 (53.6)	9 (32.1)	28
Trimethoprim	-	-	28 (100)	28
Total	18 (8)	56 (25)	150 (67)	224

Of the total isolates, *S.* Typhimurium and *S.* Haifa were multi drug resistant serovars. Of the six isolates, one isolate of *S.* Typhimurium was resistant to chloramphenicol, streptomycin and tetracycline. Two isolates of *S.* Haifa were resistant to ampicillin, tetracycline and streptomycin. One untypable isolate of *Salmonella* from mesenteric lymph nodes was also multiple antimicrobial resistant to tetracycline and streptomycin.

There were serovars which were resistant only to one antimicrobial. Serovars resistant to more than one antimicrobial were resistant to a maximum of three of the tested antimicrobials (Table 11). About 53.6% (15 of 28) of the isolates were intermediate resistant to polymyxin B.

Table 10: Antimicrobial resistance patterns of *Salmonella* serovars by type of samples

Sample type	Serovar	Number of serovars		Antimicrobial resistance pattern
		Tested	Resistant	
Liver	*S.* Typhimurium	1	-	-
	S. Mishmarhaemek	1	-	-
MLN	*S.* Typhimurium	2	1	Chl, Str, Tet
	S. Newport	1	1	Str
	S. Infantis	1	-	-
	S. Haifa	1	1	Amp, Str, Tet
	Untypable	1	1	Str, Tet
IC	*S.* Typhimurium	1	-	-
	S. Newport	3	2	Str
	S. Infantis	2	-	-
	S. Haifa	1	1	Amp, Str, Tet
	S. Heidelberg	2	2	Str
	S. Mishmarhaemek	1	-	-
	Untypable	1	-	-
CS	*S.* Typhimurium	2	-	-
	S. Newport	2	1	Str
	S. Infantis	2	-	-
	S. Haifa	1	1	Str
	Untypable	2	-	-
	Total	28	11	

Four types of antimicrobial resistance patterns were identified: Str, Str Tet, Chl Str Tet and Amp Str Tet (Table 11). *Salmonella* Mishmarhaemek and *S.* Infantis were susceptible to all of the tested antimicrobials while all isolates of *S.* Haifa and *S.* Heidelberg were resistant to streptomycin. Two isolates of *S.* Haifa were multiple antimicrobial resistant to ampicillin and tetracycline additional to streptomycin. Four of the six isolates of *S.* Newport were resistant to streptomycin.

Table 11: Prevalence of serovars by their antimicrobial resistance patterns

Type of serovar	Number of serovars	Type of antimicrobial resistance pattern			
		Str (%)	StrTet (%)	Chl Str Tet (%)	Amp Str Tet (%)
S. Typhimurium	6	-	-	1 (16.7)	-
S. Newport	6	4 (66.7)	-	-	-
S. Infantis	5	-	-	-	-
S. Haifa	3	1 (33.3	-	-	2 (66.7)
S. Heidelberg	2	2 (100)	-	-	-
S. Mishmarhaemek	2	-	-	-	-
Untypable	4	-	1 (25)	-	-
Total	28	7 (25)	1 (3.6)	1 (3.6)	2 (7.1)

5. DISCUSSION

5.2. Prevalence of *Salmonella*

The prevalence of *Salmonella* at animal level in apparently healthy slaughtered cattle was 7% which is in agreement with the 7.1% prevalence reported by Alemayehu *et al*. (2003) in a small scale abattoir at the Faculty of Veterinary Medicine (FVM), Debre Zeit and Dargatz *et al*. (2003) who reported overall prevalence of 6.3% from US cattle in two feed lots. However, it is less than the 14% prevalence reported by Sibhat, (2006) and much less than that of Samuel *et al*. (1980) who reported 75% prevalence, although direct comparison cannot be made between different studies (Fegan *et al*., 2005). This difference in prevalence could be associated with the type of samples examined and/or the bacteriological techniques used in detecting *Salmonella* or the difference in hygiene of dressing operations or difference in occurrence and distribution of *Salmonella* in the study population regardless of sample type and method of detection (McEvoy *et al*., 2003). Salmonellosis is more prevalent in areas with intensive animal husbandry where animals live in a confined environment (Wray and Davies, 2003), which is unlikely in the study area where no intensive animal production is practiced. The absence of concentrated feed or of ingredients of animal origin that might be contaminated by salmonellae being used as cattle feed could explain the low recovery rate of *Salmonella* from the study animals (Alemayehu *et al*., 2003). The number of sample types was also not inclusive and higher percentage prevalence could probably have been obtained if other organs had been examined (Molla *et al.,* 2006a).

As there is no effective means of detecting symptomless execrators (Stolle, 1981), all animals being examined and carrying salmonellae might have not been diagnosed. This is because animals in subclinical form might have latent infection and harbor the pathogen in their lymph nodes, or they might be carrier and eliminate the agent in their fecal material briefly or intermittently (Acha and Szyfres, 2001). Therefore low and intermittent fecal shedding by carrier cattle could also explain lesser chance of detecting *Salmonella* (Alemayehu *et al*., 2003). However, salmonellae which cannot be recovered on normal bacteriological media although non-recoverable remain viable and infective for animals (Jones *et al*., 2004).

Exposure of animals to predisposing factors as unhygienic conditions and overcrowding leads to shedding of *Salmonella* by carrier animals (Venter *et al*., 1994). However, it is unlikely that cattle in this study were held for a long period before slaughter, might explain the lower prevalence found in the current study (Fegan *et al*., 2005).

In this study *Salmonella* was detected both from liver, mesenteric lymph nodes and intestinal content. Carrier animals harbor organisms in their mesenteric lymph nodes; gut associated lymphoid tissue, macrophages in the lamina propria of the intestine, and the gall bladder (Venter *et al*., 1994). The prevalence of *Salmonella* in the current study had considerable variation between different sample types. The highest sample prevalence (5.9%) was detected from intestinal content while the lowest prevalence (1.1%) was detected from liver tissue samples. *Salmonella* serovars varies in various parts of animals (Jay, 2000).

The prevalence of *Salmonella* in the intestinal content (5.9%) of this study was in agreement with 6% prevalence of *Salmonella* from caecal content in slaughtered cattle at a commercial slaughterhouse in Debre Zeit, Ethiopia (Sibhat, 2006). However, it was higher than prevalence of 2.1% and 2.2% reported by Pegram *et al*. (1981) and Nyeleti *et al*. (2000) respectively from fecal cultures of cattle slaughtered at Addis Ababa abattoir and 2% prevalence reported from bovine feces at a commercial abattoir in the Republic of Ireland (McEvoy *et al*., 2003). Alemayehu *et al*. (2003) also reported a prevalence of 3.1% from cattle feces in Debre Zeit, Ethiopia.

There was slight difference in prevalence of *Salmonella* between intestinal content, in this study, and caecal or fecal prevalence in other studies is that detecting salmonellae from the intestine might give more chance of isolating the organism before it gets diluted as the result of the fecal accumulation in the ceacum or higher probability of getting salmonellae as the result of their multiplication in the intestine for spread. The primary sites for invasion of animals by salmonellae are gastrointestinal tract perhaps the intestine (Samuel *et al*., 1980) probably the distal ileum from the distribution of infection. This may be because the intestinal contents are held within the distal ileum for some time before entry into the caecum and it may also be partly due to the relatively high number of M cells (specialized epithelial cells overlaying the payer's patches) in the distal ileum (Jones *et al*., 2004). Samuel and his colleagues' also found that salmonellae confined to the

gastrointestinal tract and the mesenteric lymph nodes in the 21 of 28 animals examined except in three isolates (Samuel *et al*., 1980). However, they might also be found in other parts of the body from time to time (Wray and Davies, 2003).

Mesenteric lymph nodes are considered as reservoir of *Salmonella* (Moo *et al*., 1980). Therefore culture of *Salmonella* from mesenteric lymph nodes is the principal procedure used in identifying animal carriers of *Salmonella* (Acha and Szyfres, 2001). In this study 186 mesenteric lymph node samples had been cultured and prevalence of 3.2% *Salmonella* was found. This was in agreement with 2.1% (Pegram *et al*., 1981) and 4.2% (Nyeleti *et al*., 2000) in their study at Addis Ababa abattoir, 2.4% from mesenteric lymph nodes of slaughtered animals in Debre Zeit and Addis Ababa (Molla *et al*., 2003), and 4.5% in Debre Zeit (Alemayehu *et al*. 2003). However, it was lower than 8% (Sibhat, 2006), and much lower than the findings of Samuel *et al*. (1980) and Moo *et al*. (1980), who found prevalence of 57.1% and 30% respectively from mesenteric lymph nodes of cattle in two different commercial abattoirs in Australia. The low level of prevalence in mesenteric lymph nodes in the current study might be either the organisms were present in too small to be detected by our methods as there are different detection methods of *Salmonella* with various sensitivities (Blackburn, 1993; Maddox, 2003), or else there is difference in distribution of the organisms in the different study cattle populations.

The detection of two isolates in liver (1.1%) in this study is too small to conclude whether the occurrence of *Salmonella* is due to infection of the liver or it is due to contamination from the intestinal content and/or mesenteric lymph nodes of the same animal or from another animal slaughtered on similar day or from other sources during the slaughtering operation. Liver and spleen are usually free of *Salmonella* at slaughter, but the surfaces can be contaminated during processing. 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*., 1980).

The prevalence of *Salmonella* from carcasses in our study (4.8%) was in agreement with prevalence of *Salmonella* (2%) from carcass swabs in slaughtered cattle at a commercial slaughterhouse in Debre Zeit (Sibhat, 2006) and prevalence of 3.1% and 2.8% from muscles of the diaphragm and the abdomen respectively from cattle slaughtered in a small scale abattoir at FVM, Debre Zeit (Alemayehu *et al*., 2003). Stolle (1981) found a

prevalence of 4.3% in carcass swabs taken from slaughtered cattle in Berlin, Germany. Fegan *et al*. (2005) found carcass contamination of 2% from an abattoir in Australia before chilling of carcass. It was; however, lower than 9.8% and 11.9% prevalence found from abdominal and diaphragmatic muscles respectively from cattle slaughtered at Addis Ababa abattoir (Nyeleti *et al*., 2000). McEvoy *et al*. (2003) was also found 7.6% prevalence in their study in the Republic of Ireland. The similarities and differences in carcass contamination levels have to be taken with caution as there are various facts which can affect the contamination level of carcass such as hygienic status during flaying, evisceration, treatment of carcasses before chilling, etc. For example Bacon *et al*. (2002) found a level of 1.3% beef carcass contamination after a number of decontamination measures were taken, to reduce the bacterial load of carcasses, so cannot be compared as no decontamination measures were undertaken in the current study.

The level of *Salmonella* prevalence in animals was not high to result in high level of contamination in the present study; however the presence of even a single carrier animal can be a potential source of contamination of the carcasses, environment or personnel (Abouzeed *et al*., 2000). The level of bacterial contamination associated with live animals can be amplified through slaughtering operations (Mrema *et al*., 2006) and carcasses get contaminated during the process of evisceration (Acha and Szyfres, 2001; Alemayehu *et al*., 2003) at the abattoirs (Stolle, 1981). When lymph nodes from infected animals are incised during meat inspection, a substantial reservoir of *Salmonella* would be expected and be transferred to other parts of the carcass via equipment or personnel (Samuel *et al*., 1980).

All of the 9 *Salmonella* positive carcasses (4.8%) were derived from animals from which *Salmonella* had been isolated indicate high level of contamination. This high rate of contamination, compared to animals found carrying *Salmonella* in current study indicates unhygienic status of the slaughterhouse and slaughtering operations. The source of contamination of the carcass might not be in contact with intestinal content, mesenteric lymph nodes or liver tissue of the same animal, rather it could be due to other slaughtered animals, the slaughtering environment or personnel within the abattoir (Samuel *et al*., 1980). However, these samples were not tested in this study thus no conclusions can be made whether they were responsible for the contamination of the carcasses.

Salmonella was present in the intestine, mesenteric lymph nodes, and liver of slaughtered cattle at Bahir Dar abattoir. Therefore, transfer of contamination from these organs can occur, however, an alternative source, such as the hide, or adjacent carcasses on the line, may also contribute to carcass contamination. Hence the consumer should be aware of proper cooking of meat before consumption and there should be improvement in personal and meat hygiene in the line of meat production at Bahir Dar abattoir to reduce the risk associated with meat and meat products for human consumption.

5.2. Distribution of *Salmonella* serovars

In this study, twenty four specific serovars were identified from a total of 28 isolates. The 24 identified isolates consist of six different types of serovars. There was nothing unique about the type of serovars isolated in the current study, as *Salmonella* Typhimurium, *S.* Newport, *S.* Infantis, *S.* Haifa, *S.* Heidelberg and *S.* Mishmarhaemek have been previously isolated from slaughtered animals, animal products and human in Ethiopia (Pegram *et al*., 1981; Tibaijuka *et al*., 2003; Molla and Mesfin, 2003; Alemayehu *et al*., 2003; Ejeta *et al.*, 2004; Zewdu, 2004; Molla *et al*., 2004; Woldemariam *et al*., 2005; Molla *et al.,* 2006a; Molla *et al*., 2006b; Sibhat, 2006; Aragaw *et al*., 2007).

Some serovars were more prevalent than others in this study. *Salmonella* Typhimurium and *S.* Newport were the most frequently isolated serovars, each accounting for 21.4% of the total isolates. *Salmonella* Typhimurium was previously reported from fecal cultures of slaughtered cattle at Addis Ababa abattoir as dominant serovar accounting 5 of the 10 isolates (Pegram *et al*., 1981). Alemayehu *et al*. (2003) also reported that *S.* Typhimurium was the second dominant serovar accounting 20% of their total isolates from cattle in Debre Zeit. *Salmonella* Typhimurium was also reported being the dominant serovar from apparently healthy slaughtered sheep and goats with prevalence of 25% and 39.8% respectively (Molla *et al*. 2006a). In beef cattle presented at slaughter in Australia, Fegan and his colleagues reported *S.* Typhimurium as the dominant serovar in their isolates (Fegan *et al*., 2004). Even though it was not dominant in his isolates, Sibhat (2006) reported *S.* Typhimurium from slaughtered cattle at commercial abattoir in Debre Zeit with a prevalence of 1.1%.

Salmonella Newport had been isolated from slaughtered cattle at commercial abattoir in Debre Zeit as the second dominant serovar accounting 20.7% of the total isolates (Sibhat, 2006). Apart from their occurrence in slaughtered cattle, *Salmonella* Typhimurium and *S.* Newport had also been reported in Ethiopia from other animal species and their byproducts (Tibaijuka *et al*., 2003; Molla and Mesfin, 2003; Molla *et al*., 2004; Woldemariam *et al*., 2005; Aragaw *et al*., 2007). *Salmonella* Newport was previously reported from a variety of food items with prevalence of 13.8%, 54.5%, 52.2%, 25%, 75%, 66.6%, and 100% of all the *Salmonella* isolates from chicken meat, pork, mutton, minced beef, local cheese, fish and stool samples respectively from supermarkets and their personnel in Addis Ababa (Zewdu, 2004). This wide distribution of occurrence and dominant frequency of *Salmonella* Typhimurium and *S.* Newport in this and previous studies indicate public health concern, as these organisms may reach the consumer along the production chain, which become more serious in Bahir Dar in particular and in Ethiopia in general as the tradition of consuming raw or undercooked meat is common.

Salmonella Infantis was isolated with prevalence of 17.9% of the total isolates in the present study which was less than the report of Ejeta *et al*. (2004) who reported a prevalence of 43.5% *S*. Infantis and as dominant serovar in their study from minced beef in Addis Ababa abattoir. Zewdu (2004) reported one isolate of *Salmonella* Infantis from minced beef in his study of food items and personnel at Addis Ababa. Prevalence of *Salmonella* Infantis had also been reported from different animal species, Molla *et al*. (2004) from camel, Ejeta *et al*. (2004) from mutton and pork and Woldemariam *et al*. (2005) from sheep and goats in Ethiopia and Dargatz *et al*. (2004) from cattle in abroad indicated its distribution and the potential public health risk associated with this serovar.

Salmonella Haifa was the other isolated serovar with prevalence of 10.7% in the current study. Because there is no any previous report of *S*. Haifa in cattle in Ethiopia it was impossible to compare the relative occurrence of this serovar in Bahir Dar and other previous study sites in Ethiopia. However it was previously reported by Tibaijuka *et al*. (2003) with relatively equivalent prevalence of 6.7% from raw chicken products.

Salmonella Heidelberg and *S.* Mishmarhaemek were detected with equal level of prevalence (7.1%) in this study. The prevalence of *S.* Mishmarhaemek in this study was much less than prevalence of 48% reported in previous study from apparently healthy

slaughtered cattle at Debre Zeit (Alemayehu *et al*., 2003). *Salmonella* Heidelberg was also reported as it contributed 4 of 8 isolates from sheep in a study at Modjo abattoir in apparently healthy slaughtered sheep and goats of central Ethiopia (Molla *et al*., 2006a).

In general the similarities of serovars between the different sample types indicate contamination during the slaughtering process either from the slaughtered animals themselves or from other common sources. Of course the possibility of detecting similar serovars from different sample types due to the distribution of these serovars in the study area should not be ignored. When the current study was compared to other previous studies there was marked difference in the type and frequency of serovars in food animals including cattle and in food of animal origin. This may in part be due to the difference in distribution of *Salmonella* serovars among the different animal hosts or due to the limited number of samples analyzed. However, it is impossible to predict why the frequency and importance of a serovar will change with time (Lax *et al*., 1995), which is a future area of research.

5.3. Antimicrobial resistance patterns

In the present study, antimicrobial susceptibility test were undertaken on 28 *Salmonella* isolates to a panel of eight antimicrobials. The purpose of surveillance of antimicrobial resistance is to detect shifts in susceptibility of various organisms to various antibacterial agents and to inform prescribers and other interested parties of such changes as soon as possible. If an increase in resistance is noted in any species, information from surveillance studies can help to direct appropriate therapy to treat emerging resistant pathogens and to allow strategies to be formulated aimed at reducing or preventing any further development of resistance (Bax *et al*., 2001), or in the development of antimicrobial analogs that will be useful in the treatment of drug resistance organisms (Mills- Robertson *et al*., 2003).

Out of the 28 isolates tested, 11 (39.3%) were resistant to one or more of the tested antimicrobials. This result is comparable with 41.4% resistance reported for isolates from cattle (Sibhat, 2006). However the level of resistance in this study was higher than 31.8% and 31.9% overall resistance reported for isolates from slaughtered sheep and goats (Molla *et al*., 2004) and from swine (Aragaw *et al*, 2007) respectively. It was also lower than

44.8% resistance reported for isolates from camels (Molla *et al*., 2004) and much lower than the prevalence of antimicrobial resistance (57% to 64%) for one or more antimicrobials reported from Ethiopian chicken carcass and giblets (Tibaijuka *et al*., 2003; Molla *et al*., 2003). In Ethiopia, high level of antimicrobial resistance (93%) has also been reported in human from diarrheal out-patients (Mache *et al*., 1997; Mache, 2002).

When animals from which they originate have been treated with the particular antimicrobial over a long period, transmitted bacteria will have acquired resistance to specific antimicrobials (Dargatz *et al*., 2003). Therefore, difference in the level of antimicrobial resistance from previous studies might be described from the view of differences in the frequency and type of antimicrobials used in an area where the animals originated, or might arise from differences in bacterial culture and antimicrobial susceptibility testing techniques (Padungtod and Kaneene, 2006). It might also be explained from differences in the number and type of antimicrobials used in different studies.

In the present study the lowest resistance pattern was observed against one antimicrobial while the highest was observed to three antimicrobials. Resistance to more than one antimicrobial was observed in 14.3 % (4 of 28) of isolates. This was by far lower than multiple antimicrobial resistant of *Salmonella* isolates reported from different sources in Ethiopia. Multiple antimicrobial resistant among the human antimicrobial resistant *Salmonella* isolates in Ethiopia ranged from 51.9% (Gedebou and Tassew, 1981) to 93.5 % (Mache *et al*., 1997) with results from other studies fitting between these values (Ashenafi and Gedebou, 1985; Mache, 2002). Most of the reports by different researchers on antimicrobial resistance of salmonellae in slaughtered animals and animal products in Ethiopia, indicated that the proportion of multidrug resistant isolates was higher than isolates resistant to a single antimicrobial, ranging from 54.9% (Tibaijuka *et al*., 2003) to 100% (Alemayehu *et al*., 2003; Molla *et al*., 2006a; Aragaw *et al*., 2007). Reports of multi drug resistant isolates by other investigators fall between these values (Molla *et al*., 2003; Zewdu, 2004; Molla *et al*., 2006b).

In looking the antimicrobial resistance pattern of different serovars to each of the antimicrobials, there was variation in the antimicrobial susceptibility pattern among the

different serovars. Of the six identified serovars the highest level of resistance was seen in *Salmonella* Newport, resistant isolates accounted for 66.7% (4 of 6) of the total *S.* Newport isolates and all were resistant only to streptomycin. This contradicts to previous reports on S. Newport in Ethiopia, Aragaw *et al.*, (2007) from swine and Zewdu (2004) from foods of animal origin and human beings reported all isolates of *S.* Newport were susceptible to all the tested antimicrobials including streptomycin. However, though it was not for streptomycin Sibhat (2006) also reported 20.6% resistance isolates of *S.* Newport from cattle in a commercial abattoir at Debre Zeit to tetracycline. Dargatz *et al*. (2003) reported as *S.* Newport is an emerging multiple antimicrobial resistant *Salmonell*a serovar in feedlots cattle in US. Even though less in number compared to *Salmonella* Newport all serovars of *S.* Heidelberg were resistant to streptomycin. However Molla *et al*. (2006a) reported that all isolates of *S.* Heidelberg were susceptible to all the antimicrobials from apparently healthy slaughtered sheep and goats in central Ethiopia.

All the three isolates of *S.* Haifa were resistant to streptomycin; however, the case of antimicrobial resistance pattern was different when compared to *S.* Newport and *S.* Heidelberg. Only one of the three isolates of *S.* Haifa detected from carcass swab was resistant to streptomycin alone while the rest two isolated from mesenteric lymph nodes and carcass swab samples were multiple antimicrobial resistant to ampicillin, streptomycin and tetracycline; however all the isolates were susceptible to all the rest antimicrobials tested in the panel.

Except one, all isolates of *Salmonella* Typhimurium were susceptible to all tested antimicrobials in the current study, which was in agreement with Demissie (2005) from selected samples in Debre Zeit and Addis Ababa and Sibhat (2006) from cattle in commercial abattoir at Debre Zeit, who reported all isolates of *S.* Typhimurium were resistant to his test antimicrobials which were identical to used in this study. One isolate of *S.* Typhimurium was multi drug resistant to chloramphenicol, streptomycin and tetracycline, which was in agreement with Molla *et al*. (2006a) who reported multi drug resistant *S.* Typhimurium for similar antimicrobials. Molla *et al*. (2003) and Aragaw *et al*. (2007) also reported multi drug resistant *S.* Typhimurium up to 10 antimicrobials from their respective studies.

In agreement with Aragaw *et al*. (2007) all isolates of *Salmonella* Infantis in this study were susceptible to all the antimicrobials. However, in a study from slaughter swine antimicrobial resistant S. Infantis were reported in Australia (Morgan *et al*., 1987). All isolates of *S.* Mishmarhaemek were susceptible to all the antimicrobials tested in this study like the antimicrobial resistance pattern of *Salmonella* Infantis.

In general none of *Salmonella* positive samples contained an isolate resistant to gentamycin, norfloxacin and trimethoprim while streptomycin was the least effective antimicrobial. Molla *et al*. (2006a) reported all *Salmonella* isolates were susceptible to gentamycin. However Aragaw *et al*. (2007) reported 1.2% gentamycin resistant *Salmonella* isolates from swine at Addis Ababa. Of the 28 *Salmonella* isolates tested for antimicrobial resistance 11 (39.3%) of the isolates were resistant to streptomycin which was in agreement with Molla *et al.* (2003), Sibhat (2006) and Aragaw *et al*. (2007) who reported a level of 22.5%, 24.1% and 32.4% streptomycin resistant serovars among the *Salmonella* isolates from chicken carcass, cattle and swine respectively. The second less effective antimicrobial against *Salmonella* isolates of the current study was tetracycline followed by ampicillin. Four of the 28 isolates (14.3%) were resistant to tetracycline which was in agreement with Zewdu (2004) and Molla *et al*. (2006a) who reported tetracycline resistant *Salmonella* serovars with prevalence of 15.3% and 13.6% from food items in Addis Ababa and from sheep and goats in central Ethiopia.

Infections with bacteria of intermediate susceptibility may be considered moderately susceptible and may respond to antimicrobial agents with a wide safe dosage range (NCCLS, 1997). Intermediate resistant isolates were, therefore, considered as susceptible for this discussion based on the guide line.

In recent years, antimicrobial-resistant *Salmonella* strains have been isolated with increasing frequency (Molla *et al.*, 1999; Gebreyes *et al*., 2000; Mrema *et al*., 2006). Lack of stringent regulations and monitoring in the dispensing and use of antimicrobials in veterinary establishments and mass inoculation of herds of animals by some farmers has risen as a contributory factor to increase antimicrobial resistance. The level of resistance was high to the commonly used antimicrobials (streptomycin and tetracycline) in Ethiopia. Therefore attention against the rise in resistance of salmonellae to antimicrobials is essential (Mrema *et al*., 2006). Antimicrobial resistance is a global problem, which is not restricted to specific countries or bacterial pathogens (Acha and Szyfres, 2001). However,

the problem of antimicrobial resistance is more complex and difficult in developing countries (Molla *et al*., 1999, 2006b; Aragaw *et al*., 2007) particularly in countries of sub Saharan Africa like Ethiopia. This is mainly due to the fact that in most of these countries (i) *Salmonella* and other major pathogens including zoonotic bacteria are not routinely isolated and identified, (ii) the resistance of bacterial pathogens of veterinary and public health importance including *Salmonella* to commonly used antimicrobials is rarely assessed either in public or animal health sectors, (iii) people have easy access to various antimicrobials and can purchase without prescription, and (iv) incomplete treatment courses due to patient non-compliance are common practices.

6. CONCLUSION AND RECOMMENDATIONS

In the present study *Salmonella* was detected in one or more of liver, mesenteric lymph nodes, intestinal contents and carcasses of apparently healthy slaughtered cattle at Bahir Dar abattoir Ethiopia. Of the total animals examined, 4.8% carcass contamination was observed indicating poor hygienic status of the slaughterhouse and slaughtering operations.

A total of six different *Salmonella* serovars including *S.* Typhimurium, *S.* Newport, *S.* Infantis, *S.* Haifa, *S.* Heidelberg and *S.* Mishmarhaemek were detected being S. Typhimurium and *S.* Newport dominant isolates.

All tested isolates were susceptible to the antimicrobial effects of gentamycin, norfloxacin and trimethoprim. Antimicrobial resistance was observed mainly to streptomycin followed by tetracycline and ampicillin. Both single and multiple antimicrobial resistance patterns were observed. Among the *Salmonella* serovars, resistance (single and/or multiple) was observed in *S.* Typhimurium, *S.* Newport, *S.* Haifa, and *S.* Heidelberg. This is of special concern in Ethiopia where antimicrobials are indiscriminately used both in the veterinary and public health sector. Therefore, the following recommendations are forwarded:

- Measures should be taken at the slaughterhouse in order to reduce contamination of red meat and other edible offals during the slaughtering operations.

- Responsible and prudent use of antimicrobials should be in place in the veterinary sectors in the study area.

- Consumer awareness and proper cooking of meat and meat products should be practiced to reduce the risk of salmonellosis and other food born pathogens.

- Further detailed studies involving different abattoirs and different types of food animals on the contamination levels, diversity of serovars and antimicrobial resistance of *Salmonella* is recommended in the study area.

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8. APPENDICES

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

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

Composition (g/liter):
Peptone from casein 10.0; sodium chloride 5.0; di-sodium hydrogen phosphate 3.5; potassium dihydrogen phosphate1.5.

Preparation: Twenty grams of this medium was dissolved in one liter of distilled water and sterilized by autoclaving at 121^{o}C for 15 minutes.

2. Rappaport-Vassiliadis with Soya (RVS) (Titan Biotech, Raj, India)

Composition (g/liter):
Magnesium chloride (anhydrous) 13.58; Sodium chloride 7.2; Soya peptone 4.5; potassium dihydrogen phosphate 1.26; Di- potassium hydrogen phosphate 0.18; Malachite green 0.036.

Preparation: Suspend 26.75 grams of the powder in 1 liter of purified water. Gently heat to dissolve the medium completely. Dispense 10 ml amounts into tubes and sterilize by autoclaving at 115^{o}C for 15 minutes.

3. Muller-Kauffmann tetrathionate novobiocin (MKTTn) broth (Oxoid, Hampshire, England)

Composition (g/liter):
Meat extract 0.9; peptone from meat 4.5; yeast extract 1.8; sodium chloride 4.5; calcium carbonate 25; sodium thiosulphate 40.7; ox bile, dried 4.75; potassium iodide 5; iodine 4; brilliant green 0.01.

Preparation: Suspend 82 grams of the powder in one liter of distilled water, heat briefly to boil cool rapidly. Don't autoclave. Potassium iodide solution 20ml/lt and a0.015 solution of brilliant green (10 ml/liter) dispense into test tubes taking care to suspend any precipitate evenly. Preparation of the iodine/potassium iodide solution is potassium iodide 5 gm; iodine 4 gm; distilled water 20 ml. The ready-to-use broth should be prepared and used on the same day.

4. MacConkey Agar (Titan Biotech Limited, Bhiwadi, India)

Composition (g/liter):
Peptone 17.0; protease Peptone 3.0; lactose 10.0; bile salts 1.5; sodium chloride 5.0; neutral red 0.03; agar 15.0.

Preparation: Suspend 51.5g in 1 liter of distilled water. Dissolve by stringing the powder completely. Sterilize by autoclaving at 121 ^{O}C for 15 minutes poured into petridishes.

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

Composition (g/liter):
Yeast extract 3.0; L-lysine hydrochloride 5.0; xylose 3.75; lactose 7.5; sucrose 7.5; sodium deoxycholate 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)

Composition (g/liter):
"Lab-Lemco" powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0.
Preparation: Suspend 28g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121^{o}C for 15 minutes.

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

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

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

8. Lysine iron agar (LIA) (Difco™, Becton Dickinson, Claix, France)

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

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

9. Urea agar base (BBL®, Becton Dickinson, USA)

Composition (g/liter):
Pancreatic Digest of Gelatin 1.0; dextrose 1.0; sodium chloride 5.0; potassium phosphate 2.0; Urea 20; Phenol red 0.012.
Preparation: Suspend 29g of the powder in 100 ml of distilled water. Mix thoroughly and sterilize by filtration. Suspend 15g of Agar in 900 ml distilled water. Autoclave at 121^{o}C for 15 minutes. Cool to 50^{o}C and add 100 ml of filter sterilized urea agar base. Mix thoroughly and dispense aseptically in sterile tubes. Cool tubed medium in a slanted position so that deep butts are formed.

10. SIMMON'S CITRATE AGAR (Difco, Detroit, USA**)**

Composition (gram/litter):
Magnesium sulphate 0.2g, ammonium dihydrogen sulphate 1.0, dipotassium phosphate 1.0, sodium citrate 2.0, sodium chloride 5.0, Bacto agar 15.0, Bacto bromothymol blue 0.08.

Preparation: Suspend 24.2g in 1 litter of distilled or deionized water and boil to disolve completely. Dispense in to final containers and sterilize at 121-124oc for 15 minutes. Cool tubed medium in a slanted position so that deep butts are formed.

11. **Rambach®-agar** (Merck, Darmstadt, Germany)

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

Preparation:
i. One vial of liquid mix was added to 250 or 1000 ml distilled water and mixed by swirling until completely dissolved (The water quantity is dependent on the respective pack size.)
ii. One vial of nutrient-powder was added and mixed by swirling until completely suspended.
iii. The medium was heated in a boiling water bath until totally dissolved, while carefully shaking from time to time.
iv. The medium was cooled as fast as possible in a water-bath (45-50 ^{O}C). During this procedure (max. 30 minutes) it gently shook from time to time and poured in to plates.
v. In order to prevent any precipitate or clotting of the chromogenic-mix in the plates, Petri dishes were placed on a cool surface during pouring procedure.

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

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

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

13. **Tryptone Soya broth** (Oxoid, England)

Composition (g/liter):
Pancreatic digest of casein 17.0; pancreatic digest of Soya bean meal 2.0; Sodium chloride 5.0; Di-basic potassium phosphate 2.5 and Glucose 2.5.

Preparation: Dissolve 30.0g in one liter of distilled water and distributed into final containers, sterilize by autoclaving at 121 ^{O}C for 15 minutes.

14. **Muller-Hinton Agar** (Difco, Becton Dickinson, Claix, France)

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

Preparation: Dissolve 34.0 g in 1 liter of distilled water, boil to homogenize it, autoclave at 115^{o}C for 10 min, cool to 50^{o}C and dispense on to sterile petridishes.

Appendix 2: Preparation of 0.5 McFarland Turbidity Standard.

Solution A (0.048 M $BaCl_2$):

1.175 g $BaCl_2.2H_2O$

Make up to 100ml with distilled water.

Solution B (0.18M H_2SO_4)

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

Make up to 100 ml with distilled water

For standard:

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

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

9. CURRICULUM VITAE

A. Biographical Data:

Name	Sefinew Alemu Mekonnen
Date of birth	August 1, 1973.
Place of birth	East Gojam, Ethiopia
Marital status	Married
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Veterinarian in North Wollo Zone of Amhara National Regional State

B. Educational background

Year	Institute	Achievement
1980- 1985	Awuja elementary school, East Gojam	Completion of primary and junior secondary school
1986	Debre Work junior secondary School, East Gojam	
1987	Gundewoin junior secondary, East Gojam	
1988 - 1991	Motta senior Secondary School, East Gojam	Completion of secondary school
1992	Bole senior Secondary School, Addis Ababa	
1993	Addis Ababa University, Faculty of Science	Completion of freshmen program
1994 -1999	Addis Ababa University, Faculty of veterinary medicine	Doctorate degree in Veterinary Medicine

C. Work Experience

November 1999 to August 2004 Government employed field veterinarian in north Wollo Zone of Amhara National Regional State.

D. Research output/Technical paper

Study on small ruminant lungworms in north-eastern Ethiopia. **Sefinew Alemu**, Esayas Gelaye Leikun, Gelagay Ayelet, and Aschalew Zeleke (2006) *Veterinary Parasitology*, **142**, 330-335.

E. Membership to professional societies

Member of Ethiopian Veterinary Association.

F. Language

Amharic	Mother tongue
English	Writing and speaking

G. Computer Skill

MS Dos, MS Word, MS Excel

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10. SIGNED DECLARATION SHEET

This thesis is 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 (DVM, MSc, PhD. Assoc. Prof.) _____