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# EPIDEMIOLOGICAL STUDY AND ZOONOTIC IMPORTANCE OF BOVINE SALMONELLOSIS IN SELECTED SITES OF ADDIS ABABA, ETHIOPIA

by
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ADDIS ABABA UNIVERSITY AND FREIE UNIVERSITÄT BERLIN

# EPIDEMIOLOGICAL STUDY AND ZOONOTIC IMPORTANCE OF BOVINE SALMONELLOSIS IN SELECTED SITES OF ADDIS ABABA, ETHIOPIA

A thesis submitted in partial fulfilment for the degree of Master of Science in Tropical Veterinary Epidemiology at the Freie Universität Berlin and Addis Ababa University

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**Dedication**

**My special dedication to my beloved father, my wife Irene and son Adrian for the spiritual support at the time I was away from them. My late mother M.H.S.RI.P.**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AVID | Arbeitskreis für veterinärmedizinische Infektionsdiagnostik |
| BgVV | Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin |
| BPLS | Brilliant Green Phenol-Red-Lactose-Sucrose agar |
| BPW | buffered peptone water |
| CI | confidence interval |
| CCIS | computer coding and identification systems |
| DIN | Deutsches Institut für Normung |
| FAO | Food and Agriculture Organisation |
| G | gram |
| GDP | gross domestic product |
| GMP | Good Manufacturing Practices |
| GPP | Good Production Practices |
| H | hour(s) |
| $H_2S$ | hydrogen sulphide |
| ICMSF | International Commission on Microbiological Specifications for Foods |
| ISO | International Organisation for Standardization |
| l | liter |
| LL | lower limit |
| LPS | Lipopolysaccharide-Protein Complex |
| µl | microliter |
| NA | nutrient agar |
| NaCl | sodium chloride |
| NMCA | National Meteriological Services Agency |
| RV | Rappaport-Vassiliadis medium |
| SC | Selenite Cystine |
| sp. | species |
| subsp. | subspecies |
| TSI | Triple Sugar Iron |
| Tm | temperature |
| UL | upper limit |
| VP | Voges-Proskauer |
| VPH | Veterinary Public Health |
| WHO | World Health Organisation |
| XLD | Xylose-Lysine Desoxycholate Agar |

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## ABSTRACT

A cross-sectional study was carried out for the period of 6 months in selected sites of Addis Ababa in order to determine the prevalence and distribution of *Salmonella* in the cattle chain from abattoir to the consumer. The sources analyzed were pooled cattle faeces, pooled mesenteric lymphnodes, single muscles from the diaphragm as well as from the abdominal region. Additional minced beef from supermarkets destined for human consumption and additinally stool samples from the abattoir personnel were included in the survey. The isolated *Salmonella*–strains were serotyped and resistance against antibiotics determined.

For the culture method, the technique recommended by the International Organization for Standardization (ISO 6579, 1998) was chosen. Antimicrobial results were analyzed based on the descrption by AVID (1987). All the data collected on the prevalence and *Salmonella* distribution from the samples were presented in form of tables and figures. The Chi- Square test analyzed statistically whether there was association between *Salmonella* prevalence with animal condition or mode of transport conditions, supermarkets or storage conditions in supermarkets.

Analyzing prevalence and distribution of *Salmonella* from 235 abattoir cattle randomly selected samples leads to the following results: in 47 pooled samples of faeces (containing five samples) 5 *Salmonella* strains (10.6 %) were isolated, in 47 pooled samples of mesenteric lymphnodes there were 9 strains (19.6 %). In 235 single samples of the abdominal (oblique and transverse muscle) 23 (9.8 %) and in 235 diaphragmatic muscles 28 *Salmonella* were found (11.9 %). From 300 stool samples of the abattoir personnel in 18 cases (6.0 %) *Salmonella* were isolated. Minced beef from the 22 supermarkets with an overall of 330 samples contained in 26 of the units (7.9 %) *Salmonella* cases.

In the entire study, 98 surviving *Salmonella* strains were serotyped and identified as 27 *S.* Anatum, 53 *S.* Dublin, 5 *S.* Meleagridis, 1 *S.* Muenchen, 9 *S.* Saintpaul *and* 3 *S.* rough form. From the pooled samples of faeces 4 *S.* Dublin and 1 *S.* Muenchen, of the pooled samples of mesenteric lymphnodes 3 *S.* Anatum and 2 *S.* Dublin have been found. In single samples of abdominal muscles 18 *S.* Dublin and 3 *S.* Anatum and in diaphragmatic muscle samples 21 *S.* Dublin and 2 *S.* Anatum occurred. The cattle specific *S.* Dublin was the most dominant serovar in all the cattle samples. The 18 *Salmonella* isolates from the abattoir personnel consisted of 7 *S.* Aantum, 4 *S.* Dublin, 5 *S.* Meleagridis and 2 *Salmonella* rough

forms. In this case *S. Anatum* was the most predominant serovar followed by *S. Meleagridis* which was never isolated from the cattle chain. From minced beef 26 *Salmonella* strains were cultivated comprising 12 *S.* Anatum, 4 *S.* Dublin, 9 *S.* Saintpaul and 1 *S.* rough form. 3 *Salmonella* serotypes, (2 *S.* Anatum, 1 *S.* Dublin and 6 *S.* Saintpaul) originated from Kaliti abattoir. In this of Kara abattoir all 3 strains belonged to *S.* Saintpaul. In minced meat originating from Addis Ababa 10 *S.* Anatum, 3 *S.* Dublin and 1 *S.* rough form but no *S.* Saintpaul were found. In all the samples of Addis Ababa abattoir (faeces, mesenteric lymphnodes, beef cuts and minced meat) no *S.* Saintpaul was detected neither.

The 98 *Salmonella* isolates were tested for resistance against the seventeen antibiotics. This result is an indication of rarely using antimicrobial agents in Ethiopian cattle, hence no drug resistance was observed during the antimicrobial tests.

Low prevalence of *Salmonella* in the living cattle, indicated by low isolation rates from faeces and lymphnodes, at one hand and high *Salmonella* prevalence in samples of beef cuts (abdominal and diaphragmatic muscles) on the other hand lead to the conclusion that there must have been severe contamination during the skinning process as a result of bad hygiene during subsequent dressing operations. To reduce the *Salmonella* burden to the public it is therefore essential to improve the hygienic conditions at slaughterhouse and to slaughter only health animals. In future there should be planned a study contaning all the meat products with a considered *Salmonella* contamination risk to quantify the total hazard of *Salmonella* infection for the consumser.

# 1. INTRODUCTION AND OBJECTIVES

Ethiopia is found in the eastern part of Africa and is situated between Eritrea in the north-east, Djibouti in the east, in the south-east Somalia, Kenya in the south-west and in the west and north-west Sudan. The area of the country is 1.128.176 $km^2$ (Microsoft Encarta, 1994).

The country has an estimated human population of about 60 million inhabitants (1999 estimates). Agriculture accounted for 48 % of the GDP in 1992, and engaged 73 % of the economically active population. The economy of Ethiopia depends heavily on the earnings from the agricultural sector. The country has the highest livestock population in Sub-Saharan Africa with an estimated numbers of 31 million cattle, 24 million sheep, 18 million goats, 7 million equines, 0.63 million mules, 5.2 million donkeys, 1.07 million camels and 52 million chickens (FAO, 1993). Over 60 % of the cattle and sheep are found in the highlands.

The livestock population of Addis Ababa is estimated to be 58.568. Livestock species found are cattle, sheep, few goats, horses and donkeys. The predominant species is cattle. Holstein-Friesians, Holstein-Friesian crosses with Zebu and Zebu breed types are available. They are mainly kept for milk and meat. Zebu oxen are used for ploughing. The livestock management system varies between intensive (dairy), semi-intensive and extensive (Microsoft Encarta, 1994).

As a matter of principle salmonellae have been found in all domesticated mammals and the environment (Sojka et al.,1970). Microbial foodborne infections and intoxications in human beings are commonly associated with microorganisms of animal origin (Addo and Diallo, 1981). Consumption of contaminated meat and meat products have been responsible for many of these foodborne diseases (Bryan, 1980). Such contamination occurs within slaughterhouses, during processing and handling of meat prior to sale (Addo and Diallo, 1981). Foodborne disease caused by *Salmonella* is a very important public health problem, not only in developed countries but also in developing ones. The hazard occurs mainly due to changes in nutritional habits, the manner in which foods are marketed and because of deficiencies during production, storage and distribution. This situation has led to serious economic losses in public health and food industry (WHO, 1993).

Salmonellosis occurs in cattle from intensive, semi-intensive and extensive rearing practices, use of contaminated feeds and cross contamination of carcasses during slaughtering operations. Environmental factors such as insects, rodent and avian vectors have also been involved the infection of herds and contamination of processing areas (WHO, 1993). *Salmonella* infection in calves can be fatal, whereas infection in adult animals tends to be limited to a healthy carrier state. Stress associated with transport of animals from rearing farms to abattoir augments shedding of salmonellae (Samuel et al., 1981). Crowding and prolonged lairage in abattoir pens predisposes animals to infection. In some studies abattoirs have demonstrated as potential sources of contamination of meat destined for human consumption. Several reports have stressed the impact of poorly disinfected knives and other slaughtering equipment, and poor hygiene among plant personnel on carcass contamination. One study revealed that the hands of workers in meat processing plants generally carried 6-15 salmonellae per hand and up to 140 salmonellae per hand of slaughterhouses personnel and a subsequent study reported even higher numbers on the hands of workers in the abattoir (WHO, 1994).

Salmonellosis is known to exist in Ethiopia in animals as well as in humans. The distribution of the disease is not well known except that the causative agent has been isolated from poultry and humans in Ethiopia (Molomo, 1998; Mache and Mengistu, 1998). Forty-five *Salmonella* strains were isolated from 700 samples collected from adult diarrhoeal out patients in Addis Ababa. Among the isolates, comprised sero group C 31.1 %, B 24.4 %, *S.* Typhi 15.6 %, D 13.3 %, A 8.9 % and E 6.7 %. Studies conducted in Uganda showed, *Salmonella* organisms occurred in the stools of 8.1 % of the patients with acute diarrhoeal in Kampala district, and belonged to *S.* Typhimurium and *S.* Enteritidis serovars. *S.* Enteritidis human isolates were phagetype 14b, which is reported here for the first time in Africa. Among animals, *Salmonella* was only recovered from 4.8 % of slaughter pigs and belong to *S.* Bailey serotype (Nasinyama et al., 1998).

Studies carried out in Botswana, showed isolation of salmonellae from 5 of the 57 faeces samples from sick animals (8.8 %) and from 6 of the 89 faeces samples from apparently healthy cattle (6.7 %). Serotypes found were *S.* Anatum, *S.* Brancaster, *S.* Donna, *S.* Pomona and *S.* Typhimurium in the first group and *S.* Colorado, *S.* Enteritidis, *S.* Goodwood, *S.* Leopoldville, *Salmonella* 6,7:z:$z_6$, and one unidentified in the second group (Miller, 1971). The other serotypes encountered were *S.* Typhimurium and *S.* Fischerkietz from pooled bile samples. A total of 752 meat samples and 117 from liver samples were received from different parts of the country. The serotypes isolated from the meat were *S.* Windhoek and *S.* Offa and the liver *S.* Braenderup, *S.* Weltervreden and *S.* Newington (Miller, 1971). In Zimbabwe studies a wide range of serotypes was isolated (Chambers, 1977). The most frequent identified were *S.* Senftenberg, *S.* Newington, *S.* Mobeni, *S.* Typhi, *S.* Infantis, *S.* Typhimurium, and S. Heidelberg both in slaughter cattle and human. In Zambia the work carried out for the first time in cattle revealed 7 *Salmonella* serovars isolates these were; *S.* Amager, *S.* Bareily, *S.* Bonn, *S.* Heidelberg, *S.* Konondomi, *S.* Kisarawe and *S.* Weltervreden (Sharma et al., 1996).

Development and spread of antimicrobial resistant of *Salmonella* in food of animal origin and human has for many years constituted a special public health concern due to the increased risk of treatment failures in humans. Recently the rapid spread of strain of multidrug-resistant *S.* Typhimurium DT 204 c and *S.* Enteritidis in food of animal origin and consequently to humans, has been a cause of serious concern and is well documented (Glynn et al., 1998; Mache and Mengistu, 1998; Moloma, 1998; Malorny et al., 1999; Molla et al., 1999). Drug sensitivity tests on 23 *Salmonella* serotypes (e.g. *S.* Typhimurium, *S.* Enteritidis, *S.* Infantis, *S.* Weltvreden, *S.* Bareily and *S.* Saintpaul), isolated from milk, meat and meat products carried out using 24 different antibiotics and chemotherapeutic agents by the disc diffusion method lead to the result that the strains were were sensitive to chloramphenicol aminoglycosides, ampicillin, cephaloridine, bacitracin, polymyxin-B, cotrimoxazole and nitrofurantoin (Sharma and Joshi, 1992).

In a review of salmonellae as a foodborne pathogen, D'Aoust cited findings of studies of the *Salmonella* contamination rate of beef carcasses that varies from 0.2 %-25 % with a median of 3.3 % (D'Aoust, 1989). In a review of bovine salmonellosis, (Sojka et al., 1970) cited findings of studies of incidence of salmonellae infection in normal cattle at abattoirs ranging from 0.3 to 11.6 % but also noted that different samples and sampling techniques made the studies incomparable (Sojka et al., 1970). Isolation rates as high as 76 % from normal cattle at an abattoir have been reported (Samuel et al., 1980). The type of sample is well known to affect the sensitivity of detection, particularly in subclinically infected carrier cattle. For the detection of salmonellae infection in such cattle mesenteric lymphnodes have been reported to be superior to faeces samples, which in turn, are superior to faeces (Hardy et al., 1977).

The incidence of *Salmonella* in beef carcasses varies as mentioned above from 0.2 to 21.5 % with a median of 3.3 %, these values were derived primarily from analysis of faeces ceca, samples were received from different parts of the country. The serotypes isolated from the meat were *S.* Windhoek and *S.* Offa and the liver *S.* Braenderup, *S.* Weltervreden and *S.* Newington (Miller, 1971). In Zimbabwe studies a wide range of serotypes was isolated (Chambers, 1977). The most frequent identified were *S.* Senftenberg, *S.* Newington, *S.* Mobeni, *S.* Typhi, *S.* Infantis, *S.* Typhimurium, and S. Heidelberg both in slaughter cattle and human. In Zambia the work carried out for the first time in cattle revealed 7 *Salmonella* serovars isolates these were; *S.* Amager, *S.* Bareily, *S.* Bonn, *S.* Heidelberg, *S.* Konondomi, *S.* Kisarawe and *S.* Weltervreden (Sharma et al., 1996).

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Human salmonellosis is more widespread in young children, in elderly citizens frequently afflicted with underlying chronic diseases and in immunosuppressed individuals. Infants of one year or younger are particularly susceptible to this infection. Transmission of the infectious agent occurs primarily between humans, from various animals and environmental sources to humans, and less frequently, from humans to the environment. Demographic studies on the impact of socio-economic pressure have shown higher levels of salmonellosis in the lower socio-economic classes, and in areas with high population density level (WHO, 1985).

Considering the above facts, the current study was undertaken with the following specific objectives:

1. To estimate the prevalence and distribution of *Salmonella* in the chain from cattle to consumer, the following samples are taken:
   - faeces and mesenteric lymphnodes from cattle immediately after slaughtering,
   - muscle from the diaphragma and abdomen from slaughtered cattle,
   - mixed beef from the supermarkets destined for human consumption,
   - stool samples from the abattoir personnel.
2. To identify the most common serovars of *Salmonella* in animals and animal products as well as in humans.
3. To determine the level of antibiotic susceptibility of *Salmonella* isolates to commonly used antimicrobials.

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3. To determine the level of antibiotic susceptibility of *Salmonella* isolates to commonly used antimicrobials.

# 2. LITERATURE REVIEW

## 2.1 The Genus Salmonella

### 2.1.1 Taxonomy

Salmonellae belong to the family Enterobacteriaceae. According to the latest nomenclature that reflects recent advances in *Salmonella* taxonomy, the genus *Salmonella* consists of two species: *S. bongori* and *S. enterica* (Le Minor and Popoff, 1980). *Salmonella bongori* contains less than 10 serovars that are extremely rare. The remaining more than 2500 serovars belong to the *Salmonella enterica* species and are divided into six subspecies: S. *enterica* subspecies enterica, subspecies salamae, subspecies arizonae, subspecies diarizonae, subspecies houtenae and subspecies indica. All are distinguished by certain biochemical characteristics, some of which correspond to previous subgenera.

The genus *Salmonella* can roughly be classified into three categories or groups based on pathogenicity. The group of highly host adapted and invasive serovars includes species restricted *Salmonella* such as *S.* Pullorum, *S.* Gallinarum in poultry and *S.* Typhi in humans. The non-host adapted and invasive serovars, consist of approximately 10-20 serovars of which the most important are *S.* Typhimurium, *S.* Hadar, *S.* Arizonae and *S.* Enteritidis (Le Minor and Popoff, 1987).

### 2.1.2 Morphology

Salmonellae are gram-negative rods of following size: 2-4 µm in length and 0.5 µm in width. These organisms are facultative anaerobic and motile, except the non-motile *S.* Gallinarum. There are no morphological difference between the subspecies /serovars (Old, 1989; Seifert, 1996).

### 2.1.3 Antigenic structure

**O-antigen:** All *Salmonella* strains can be grouped to a serovar based on the analysis of somatic O-antigens. The heat-stable lipopolysaccharide-protein complex (LPC) of the cell wall contains lipid A and a core portion. The polysaccharides determine the serological specificity. About 67 O-antigens are known so far which are identified by arabic numerals 1-67. The complete O-antigen is not only the mature immunogen of the *Salmonella*. It also possesses virulence properties eliciting both humoral and cellular immune responses from *Salmonella* infection (Le Minor, 1984).

**H-antigen:** The flagellae are part of the bacterium and consist of thermolabile proteins. The specificity is determined by the pattern of amino acids. The H-antigens are designated by a combination of letters of the alphabet and numerals (Le Minor, 1988).

**K-antigen:** The hull-antigen is like a microcapsule set on the bacterial cell wall. Its own specificity may interfere with O-antigen during determination. For identification purposes, the agglutination of the O-and H-antigen is done and the results are compared with known antigenic formulae contained in the Kauffmann-White diagnostic scheme. The list is updated annually because new serovars of *Salmonella* are identified continuously (Le Minor, 1988).

In order to establish a complete antigenic composition of any *Salmonella*, serover antigens of the flagella phases as well as the "O" must be known (Le Minor and Popoff, 1997). Examples for classification by Kauffmann-White diagnostic scheme by means of agglutination test are given in table 1.

**Table 1: Classification of *Salmonella* with help of Kauffmann–White scheme showing representative serotypes (Le Minor and Popoff, 1997):**

| Group | Serovar | Somatic | Flagella antigen "H" | |
|---|---|---|---|---|
| | | | Phase 1 | phase 2 |
| B | *S.* Typhimurium | 1, 4, [15] 12 | i | 1, 2 |
| B | *S.* Saintpaul | 1, 4, [15] 12 | e, h | 1, 2 |
| C | *S.* Muenchen | 6, 8 | d | 1, 2 |
| D1 | *S.* Enteritidis | 1, 9, 12 | g, m, [f] [p] | [1, 7] |
| D1 | *S.* Dublin | 1, 9, 12 [Vi] | g, p | - |
| E | *S.* Anatum | 3, 10 [15] [15, 34] | e, h | 1, 6 |
| E | *S.* Meleagridis | 3, 10 [15] [15, 34] | e, h | 1, w |

- can only be detected in some special conditions

[ ] can be present or not.

## 2.2 Epidemiology

Homologous to the typhoid and paratyphoid infections in humans, animal host-adapted strains can adversely affect the viability and productivity of herds and flocks. The damaging effects of *S.* Dublin infections in cattle (Peters, 1985) are well documented and contrast sharply with the healthy carrier state of animals infected with non-host adapted serovars. The predominance of *S.* Typhimurium and *S.* Infantis in animal reservoirs duplicates that encountered in human population. Examination of contemporary data on the frequency of isolation of serovars from human and nonhuman sources is revealing. *S.* Typhimurium is the most common pathogen and etiological agent associated with the environment and the food chain. The prevalence of this organism in all continents including Africa, where only scant data from Uganda (Lubwana, 1985), and the Republic of Central Africa (Georges-Courbot et al., 1984) are available, corroborate designation of this serovar as a major human pathogen. Although serovars found in humans are frequently encountered in their food supply and environment, the ranking of serovars in order of frequency of isolation from the human and non-human categories may differ markedly.

## 2.3 Diagnosis

### 2.3.1 Culture

The actual principles of cultivation and isolation of *Salmonella* from routine samples are (D'Aoust, 1992; Feng 1992):

1. Non-selective pre-enrichment (16-24hours)
2. Selective enrichment (18-24 hours)
3. Isolation on selective plating media (18-24 hours)
4. Identification and serotyping

**Non-selective pre-enrichment (16-24 hours):** The optimum pre-enrichment system enables *Salmonella* to recover and protect the resuscitated cells from detrimental influences of the competitive organisms. The use of pre-enrichment provides reliable results compared to use of direct selective enrichment. Different pre-enrichment media have been described and summarised by Humbert and Colin (1991). Despite controversial discussion about the best medium, buffered peptone water (BPW) is recommended for routine examination. The optimum pre-enrichment incubation period for effective recovery of injured cells is generally agreed to be 16-24 hours.

**Selective enrichment:** The objective of enrichment media is to support the growth of target microbes present in mixture cultures. Selective enrichment media will include inhibitory substances to diminish the replication of most organisms other than salmonellae without enhancing directs the growth of the salmonellae themselves. The most common media are generally based on selenite, tetrathionate or magnesium chloride and malachite green (Vassiliadis, 1983). Peters et al. (1989) has demonstrated the importance of the incubation temperature and magnesium chloride concentration in the selective enrichment of salmonellae.

**Selective plating:** In the isolation of salmonellae, the use of suitable selective plating media increases the chances of successful isolation in the sample. Ideally, a *Salmonella* plating medium should inhibit the growth of competing microorganisms, allow the growth of *Salmonella* with characteristic colonies which could be differentiated from non-*Salmonella* spp. on the plates used. Basically, it is agreed that none of the selective plating media for *Salmonella* is ideal for all situations in the isolation of *Salmonella* in foods and that is why simultaneous use of two or more agar media is recommended almost in all *Salmonella* reference methods (Moats, 1981; Fricker, 1987; D'Aoust, 1992). There are several selective plating media for the isolation of *Salmonella*. In general terms, *Salmonella* selective plating media could be grouped into three categories depending upon the selective agents contained as: brilliant green, bismuth sulphite and bile salt agar media (Moats, 1981; Busse, 1995).

### 2.3.2 Biochemical Characterization

Salmonellae are characterised by fermentation of glucose, lysine, ornithine, adonitol, arabinose and sorbitol with the production of acid and gas; further typical reactions are deaminate and phenylalanine a positive methyl-red reaction and a negative Voges-Proskauer reaction (Andrian and Dellat, 1979). These microorganisms produce hydrogen sulphide in iron contained media and are lactose non-fermenting (Biberstein and Zee, 1990). ISO (1998) indicated that several biochemical tests may be used in the identification of to use of direct selective enrichment. Different pre-enrichment media have been described and summarised by Humbert and Colin (1991). Despite controversial discussion about the best medium, buffered peptone water (BPW) is recommended for routine examination. The optimum pre-enrichment incubation period for effective recovery of injured cells is generally agreed to be 16-24 hours.

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*Salmonella*. On triple sugar iron agar (TSI) 100 % of *Salmonella* spp. ferment glucose, and 91.9 % produce gas, 91.6 % produce hydrogen sulphide. On lysine decarboxylase 94.6 % react positive whereas 98.9 % show negative results on indole reaction and 99 % are negative for urea splitting, while 99.2 % are negative to sucrose different biochemical tests are conducted in order to differentiate the genus *Salmonella* from other related members of the family Enterobacteriaceae after growing them on selective plating media using either conventional fluid media in tubes or commercial biochemical diagnostic kits. The confirmation of the target microorganisms depends on the plating medium used and the type of colony picked for further biochemical and serological tests. The conventional tube test systems for the biochemical test of *Salmonella* are thought to be less convenient and more laborious as compared to the commercially available biochemical diagnostic kits which are effective in identifying to a genus level (Moats, 1981, D'Aoust, 1992).

### 2.3.3 Serological identification

Characterization of *Salmonella* by "O"- and "H"- antigenicity, which are thought to be essential for serovar definition, has become the most important typing method, as more than 2500 serotypes are currently recognized (WHO, 1992; Popoff et al., 1996). Serological typing is realized information an agglutination reaction and offers an effective tool for identifying the serotypes. The Kauffmann-White scheme systematically assigns numbers and letters to the different "O"- and "H"- (virulence) antigens to create distinctive serotypes (Le Minor, 1988; Quinn et al., 1994). Based on antigenic analysis, Kauffman's classification is still the most acceptable. The slide agglutination is tested with absorbed sera so that the content of "O"-antigens and phase 1 and phase 2 "H"-antigens can be determined (Seifert, 1996).

### 2.3.4 Phage typing

Phage typing is the characterization of bacteria based on susceptibility to infection by a defined panel of bacteriophages. It is the second important method to be applied in the study of *Salmonella* epidemiology. Phage types predominate in particular animal hosts. If human infections with these types happen, it can efficiently shorten the time taken to trace the animal sources of human outbreaks (Anderson et al., 1977), as well as in assisting in the control of infection in the animals themselves. Phage typing is limited to a few serovars, *Salmonella*. On triple sugar iron agar (TSI) 100 % of *Salmonella* spp. ferment glucose, and 91.9 % produce gas, 91.6 % produce hydrogen sulphide. On lysine decarboxylase 94.6 % react positive whereas 98.9 % show negative results on indole reaction and 99 % are negative for urea splitting, while 99.2 % are negative to sucrose different biochemical tests are conducted in order to differentiate the genus *Salmonella* from other related members of the family Enterobacteriaceae after growing them on selective plating media using either conventional fluid media in tubes or commercial biochemical diagnostic kits. The confirmation of the target microorganisms depends on the plating medium used and the type of colony picked for further biochemical and serological tests. The conventional tube test systems for the biochemical test of *Salmonella* are thought to be less convenient and more laborious as compared to the commercially available biochemical diagnostic kits which are effective in identifying to a genus level (Moats, 1981, D'Aoust, 1992).

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### 2.3.5 Biotyping

It is important to realize that biotyping is an essential completion to the Kauffmann-White scheme, which has been widely used to clarify the biochemical heterogeneity among *Salmonella* spp. (Ewing, 1986; Le Minor, 1988), and to segregate serovars into fermentative groups. Several biotyping schemes have been proposed for the differentiation of epidemiologically related strains of *S.* Typhimurium. The subtype (designated a-j, x, y and z) defines the pattern of reading in secondary tests. For example, biotype denotation of *S.* Typhimurium strain that is positive in the same primary (Abalaka and Dribeil, 1980) and in the 12 secondary (o) tests. This helps to establish an epidemiological relationship between strains that affect human and those that do not. Some reports on the biotyping *S.* Typhimurium from different sources and different geographical areas clearly underline the value of the biotyping scheme (Barker and Old, 1980).

### 2.3.6 Antibiogram typing

Patterns of antibiograms have been used to subgroup *Salmonella* spp. In this method, paper disks impregnated with standard amounts of antibiotics are placed on the surface of a Mueller-Hinton agar plate previously inoculated with the test organism. The zones of growth inhibition are measured following overnight incubation of the plate and resistance is assessed according to the National Committee for Clinical Laboratory Standards (1997). The typing method is rarely used alone as the typability of strains of *Salmonella* from livestock are often local due to the relatively low prevalence of antibiotic resistant strains (Olsen et al., 1993).

### 2.3.7 Molecular typing methods

The epidemiology of *Salmonella* is relatively well known due to the wide spread acceptance and use of harmonized discriminatory and definitive typing methods, notably sero- and phage typing. The molecular methods have added an extra degree of discriminatory power to the conventional methods and do allow a finer distinction between strains (Barrow, 1997). At present, typing by pulsed field gel elecrophoresis appears to be the molecular method of choice. In future, typing will most likely involve analyzing signature sequences, however these methods have not been fully developed yet. Systematic comparative molecular typing of *Salmonella* isolates from animals, food and humans can be a powerful tool to determine the public health impact of the primary sources of *Salmonella* in the country or region. Furthermore, it can provide early detection of changes in the contribution from different sources, for instance as a result of control programmes (Anonymous, 1998).

The molecular typing methods are available and in regular use, in public health and veterinary laboratories. The predominance of certain phage types of *S.* Enteritidis in various countries makes further epidemiological sub-grouping necessary. For various bacterial species, the following methodes have been proven very usefully (Helmuth and Schroeter, 1994): the plasmid profiling, the pattern of outer membrane proteins and LPS, the fingerprinting of total genomic DNA including ribotyping and multilocus enzymes electophoretic typing. When such methods have been applied to *S.* Enteritidis, they revealed a homogeneous, clonal structure in contemporary PT4 isolates. Furthermore, they indicated that the clone observed today emerged from a heterogeneous population before the onset of epidemic spreading (Helmuth and Schroeter, 1994).

## 2.4 Distribution of Salmonella serovars

### 2.4.1 Worldwide

*Salmonella* has a worldwide distribution. On the other hand the importance of *Salmonella* species differs geographically. Many serovars such as *S.* Typhimurium and *S.* Enteritidis do fail to reconginise any regional boundaries and are in sharp contrast with other serovars whose distribution tends to be restricted to certain areas. In the United States outbreaks of salmonellosis were associated with eating raw ground beef, causing gastro-intestinal illness from *S.* Typhimurium (Roels et al., 1997). Studies carried out in an abattoir in Brazil according to Campos et al. (1989), showed that the dominant serovar was *S.* Dublin from cattle (98 isolates from119 isolates of animal origin). In Finland the most common serotypes were *S.* Infantis and *S.* Typhimurium. Other serotypes were found only occasionally. In Denmark, *S.* Dublin was the predominant serotype in beef. In Germany, *S.* Typhimurium was the most frequent serotype in meat and meat products. *S.* Enteritidis was detected in varying degrees. *S.* Typhimurium was the more frequent isolated in France compared to *S.* Enteritidis ( FAO/World Bank; 1992). *S.* Uganda, as a new and highly pathogenic serovar causing food poisoning, has been isolated in human, associated with consumption of contaminated food in Italy (Ricci et al., 1990). In Iran, 45 faeces samples from diarrhoeal cows and calves contained 7 *Salmonella* serovars. 71 % of them were *S.* Enteritidis and 29 % *S.* Typhimurium. From 429 faeces samples of human suspicious to salmonellosis, 13 strains of *Salmonella* (3.03 %) were isolated, 61.5 % of them belonged to *S.*Typhi and the next two species were *S.* Paratyphi A (23.1 %) and *S.* Enteritidis (15.4 %) (Hemmatzadeh and Salemi, 1998).

### 2.4.2 Africa

Several serotypes of *Salmonella* have been reported in Africa. In Nigeria, *S.* Dublin, *S.* Widemarsh and *S.* Handoff were reported as predominant serotypes (Adesiyun and Oni, 1989). In Botswana, the most common *Salmonella* serotypes among isolates from the abattoir were *S.* Anatum, *S.* Brancaster and *S.* Typhimurium from slaughtered cattle (Miller 1971). According to Hummel (1979), in Tanzania, the most prevailing serovars were *S.* Typhimurium, *S.* Enteritidis, *S.* Gallinarum and *S.* Anatum among 436 *Salmonella* from different species of animals. These serovars are also common in Zambia (Sharma et al., 1996). In Zimbabwe, studies show a wide range of serotypes found in the abattoir cattle and personnel (Chambers, 1977). The most frequent identified serovars were *S.* Senftenberg, *S.* Newington, *S.* Mobeni, *S.* Typhi, *S.* Infantis, *S.* Typhimurium, and *S.* Heidelberg, both in slaughter cattle and human

### 2.4.3 Ethiopia

In Ethiopia, no reported studies have been carried out in the abattoir cattle. However, according to the work done by Molla et al. (1999), the common isolates from minced beef with origin of some supermarkets in Addis Ababa were *S.* Dublin, *S.* Muenchen and *S.* Anatum. *Salmonella* strains isolated from diarrhoeal patients in Addis Ababa comprised *S.* typhi with serogroups B, C, D and E (Mache and Mengistu 1998). In poultry, the isolation of *S.* Enteritidis, *S.* Uganda and *S.* Anatum causes a health risk to the public (Molomo

1998). Teshome and Mebratu (1988) have confirmed the presence of *S.* Pullorum in one broiler farm in Ethiopia. The whole range of the status of *Salmonella* serotypes existing recently is yet unknown. As today new *Salmonella* serovars infecting livestock are reported worldwide, the existence of several up to now not detected *Salmonella* serotypes is also expected in Ethiopia.

## 2.5 Public health Significance

The incidence of human salmonellosis has increased during the last decades. This increase has been associated with the spread of *Salmonella* in animal production, leading to frequent occurrence of salmonella in food for human consumption. Although nearly all *Salmonella* serotypes are potentially pathogenic to man, the two serotypes, *S.* Typhimurium and *S.* Enteritidis, predominate primarily. This fact reflects an enhanced potential in the serotypes to spread and persist in modern food of animal origin production systems (Wegener et al., 1998). Salmonellosis in human occurs after consumption of food contaminated with *Salmonella*. Additionally in most cases salmonellosis in human is influenced by factors such as age, predisposing diseases, causing *Salmonella* serovar and the infection dose. The incubation period in human is about 6-72 hours and the main symptoms are diarrhoeal, abdominal pain, vomiting, nausea, mild fever, anorexia, headaches and malaise. The infectious dose is said to be from 100 up to 10 000 bacterias. Less than 100 bacteria may cause illness in young children and in persons who are immunocompromised. Enteritis infection can also occur concurrently with immunodeficiency virus (HIV) infection and is one of the common complications of Acquired Immunodeficiency Syndromes (AIDS) (Notermans et al., 1996).

Contributing factors in episodes of foodborne salmonellosis include slow cooling of recontaminated cooked foods, inadequate cooking of raw food ingredients, improper hot storage of recontaminated cooked foods, cross-contamination of prepared foods through faulty handling of raw ingredients, or use of poorly sanitized equipment. Although use of improper holding temperatures remains the single most common fault in food handling practices, infected food handlers can also play a detrimental role in outbreaks (Bryan, 1980).

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## 2.6 Prevention and control of salmonellosis in meat and meat products of cattle

The control of *Salmonella* in beef and derived products is a most challenging task due to the complexity and interdependence of various aspects of animal husbandry, slaughtering and meat processing. Retrospective analysis of control measures and their impact on the bacterial quality and safety of meat have been disappointing because problems identified several decades ago still figure prominently in contemporary updates to the prevalence of *Salmonella* in the human food chain. However, current levels of *Salmonella* in meat products and increasing trends in human infection and food-borne outbreaks underline the need for increased vigilance and concerted government and industrial controls from the stable level to table (WHO, 1994).

Good manufacturing practices (GMP) are widely postulated to improve the bacterial quality and safety of the food supply. The success of the approach has been attained largely through stringent control of key manufacturing conditions such as temperature, pH, and bacteriological quality. The testing of raw meat and in-line control of processed meat using statistically significant sampling plans and sensitive analytical methods, as well as regular bacterial monitoring of the abattoir environment are necessary to uncover deficiencies in abattoir sanitation and to identify new sources of product contamination. (D'Aoust, 1994).

Numerous opportunities exist at the farm level to control effectively the entry and spread of *Salmonella* in farm animals. In addition to regulated importation of livestock and slaughtered animals, *Salmonella* cross-infection of herds is minimized by the use of *Salmonella* free breeding stocks and animal feeds, and the avoidance of stressful conditions arising from overcrowding of animals and from feed and water deprivation. Mainly animal feeds have been identified as an important contributor to *Salmonella* infection. The causes are processing of non-edible offal from infected animals into protein concentrates and cross-contamination of rendered products through inadequate separation of raw and finished product areas. The use of poorly designed equipment that cannot be readily dismantled and reassembled for regular disinfecting, and bulk production and shipping of feeds are some of the extra factors that contribute to the frequently poor bacterial quality of animal feeds (Brisabois, 1997).

Furthermore only meat which has been subjected to inspections according to the Codex Alimentarius Code for ante-mortem and post-mortem inspection of slaughter animals or similar provisions should be used for further processing. The ante-mortem and post-mortem inspection, however, will not necessarily detect healthy carriers, in which *Salmonella* organisms may be present in mesenteric lymphnodes or gut content. Numerous studies have shown that slaughtering of meat animals amplifies the prevalent *Salmonella* problem in farm animals. Punctual withdrawal of feeds from slaughter animals, rapid transport from the farm to the slaughtering plant, and minimal holding periods in lairage pens will substantially reduce *Salmonella* excretion and surface contamination of animal hides. Some studies demonstrated generally lower *Salmonella* contamination of raw meat products in large supermarkets than in local shops and underlined a causal relationship between frequent handling and cross-contamination of raw products (Jackson et al., 1991, Matayas, 1988).

### 2.6.1 Temperature control

In rooms where the meat is deboned, trimmed or otherwise handled with exposure to cut surfaces, the temperature should be kept sufficiently low to inhibit the growth of *Salmonella*. Studies show that some exceptional strains of *Salmonella* may grow at temperatures as low as 5.3 °C; but their growth is sufficiently retarded at 10 °C during a period of eight hours to be considered being insignificant. It is therefore advisable to carry out cutting at temperatures at or below 10 °C, provided that at the end of each working shift, normally lasting eight hours, cleaning and disinfecting of surfaces which come into contact with meat is carried out more frequently. In these rooms there should be thermometers or temperature recorders to be checked at least daily (WHO, 1994).

### 2.6.2 Cleaning of equipment

Sanitation is one of most important measure for preventing the carrying over of *Salmonella* from one operation to another or from one working area to another. Cleaning and disinfecting should be done in regular time intervals. Furthermore sanitation has to be done when an operation is changed, and under all circumstances, if raw meat has been handled on the table that will be subsequently used for the handling of cooked meat. It has been found that in a meat product establishment *Salmonella* contamination most frequently arises from sewage disposal taps, containers, filling machine, hands, walls, doors and utensils. These sources, especially those that come into direct contact with meat and meat products, are hygienic control points. Monitoring of the effect of cleaning and disinfecting should be done regularly by inspection, and occasionally, once a week, by microbiological testing with swab or agar contact methods (Matayas, 1988).

### 2.6.3 Storage and disposal of waste

It is important to ensure that waste material is handled in a way, which excludes contamination of meat, meat products and drinking water. All waste should be removed from the meat handling area, preferably immediately, but at least once a day and receptacles and the like which have been in contact with waste should be cleaned and disinfected regularly (WHO, 1994).

### 2.6.4 Processing

Meat and meat products should be prepared, processed and stored in a way which ensures that *Salmonella* organisms are inactivated to avoid contamination of the products after processing. If that precondition is not fulfilled, the multiplication of *Salmonella* must be limited. In this context the following facts on the resistance / sensitivity of *Salmonella* organisms should be kept in mind: temperatures for growth are between 5 °C and 46 °C, but below 10 °C the generation time is 10 hours or longer; pH for growth is between 4.0 and 9.0; salt (sodium chloride) concentrations which are normally used for technological or sensorical reasons will not inhibit this micro-organism. Generally, *Salmonella* like other enterobacteriacea, are not very resistant to heat. The processing of meat products must take these factors into consideration and should be supervised by competent personnel (D'Aoust, 1994).

### 2.6.5 Post-processing handling

Meat products should be effectively protected against recontamination and against growth of *Salmonella* after processing has been completed. In this respect packaging is an important factor. It may be carried out before heat processing, for instance, with cans or other containers that are heat processed at the same time as the product. The can or container sewage disposal taps, containers, filling machine, hands, walls, doors and utensils. These sources, especially those that come into direct contact with meat and meat products, are hygienic control points. Monitoring of the effect of cleaning and disinfecting should be done regularly by inspection, and occasionally, once a week, by microbiological testing with swab or agar contact methods (Matayas, 1988).

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Meat and meat products should be prepared, processed and stored in a way which ensures that *Salmonella* organisms are inactivated to avoid contamination of the products after processing. If that precondition is not fulfilled, the multiplication of *Salmonella* must be limited. In this context the following facts on the resistance / sensitivity of *Salmonella* organisms should be kept in mind: temperatures for growth are between 5 °C and 46 °C, but below 10 °C the generation time is 10 hours or longer; pH for growth is between 4.0 and 9.0; salt (sodium chloride) concentrations which are normally used for technological or sensorical reasons will not inhibit this micro-organism. Generally, *Salmonella* like other enterobacteriacea, are not very resistant to heat. The processing of meat products must take these factors into consideration and should be supervised by competent personnel (D'Aoust, 1994).

### 2.6.5 Post-processing handling

Meat products should be effectively protected against recontamination and against growth of *Salmonella* after processing has been completed. In this respect packaging is an important factor. It may be carried out before heat processing, for instance, with cans or other containers that are heat processed at the same time as the product. The can or container closure is an important control point, as imperfect sealing may cause post-process contamination (Matayas, 1988). Monitoring of can or container closures and the quality of the water used for cooling should be carried out regularly. Meat products which are heat treated prior to packaging should be promptly chilled to an internal temperature of not more than 7 °C and should be packed without delay in a separate area, where cross-contamination from raw meats can be avoided. If any operation, such as cutting or slicing, is performed before packaging, this operation should be carried out under satisfactory hygienic conditions. Packages should be regularly inspected to ensure the detection and rejection of those which are visibly defective (Matayas, 1988; WHO, 1994).

### 2.6.6 Storage and temperature of meat and meat products

Manufactured meat products should physically be separated from raw meat during storage. The storage temperature should be sufficiently below 4 °C to prevent the growth of surviving *Salmonella* in every case. Certain meat products, such as barbecued, roasted or boiled meat, alternatively may be stored at higher temperatures, e.g. in delicatessen shops or in food-service establishments (Sharp, 1990).

### 2.6.7 Sampling and laboratory control procedures

Where processing is carried out under good hygienic conditions with emphasis on the monitoring for the critical control points as outlined, it is generally not recommended to search for *Salmonella* in the end products. However, online control and the use of indicator organisms is recommended. Where there is access to an outside laboratory, meat products should be transported to the laboratory for routine or spot checks for the presence of *Salmonella*. It is advisable to carry out such checks if there are substantial changes in the processing, which might introduce conditions enabling the survival or growth of *Salmonella* (Hildebrandt and Weiß, 1994). On the basis of the "FOSTER plans" sampling procedures for detecting *Salmonella* contamination are proposed. In view of the fact that old people, children and the sick need special protection five categories were formed depending on the number of risk factors, and these categories were subsequently more closely defined. Each of these groups were associated with a particular sampling plan with (irrespective of the size of the lot) is charaterized by acceptance number c and sample size n (table 2). Plans with c =1 for cases where a positive sample is found during examination were neglected by quality control institutions and only the plans with c = 0 are used up to now.

**Table 2: Product hazard characteristics and acceptance plans (FOSTER plans)**

| |
|---|
| Category I: Non-sterile food for children, old people and sick<br>n = 60; c = 0 or n = 95; c = 1 |
| Category II: Food with three hazards characteristics, that is, sensitive ingredient, no Destructive step during manufacturer, likehood of growth if abused<br>n = 30; c = 0 or n = 48 ; c = 1 |
| Category III: Food with two hazard characteristics<br>n = 15; c = 0 or n = 24; c=1 |
| Cateegory IV: Food with one hazard charateristics<br>n = 15; c = 0 or n = 24; c =1 |
| Category V: Food with no hazard characteristic; does not usually need to be controlled<br>n = 15; c = 0 or n = 24; c = 1 |

Later on sampling plans in microbiology quality control in foods are developed by the ICMSF (International Commission on Microbiological Specifications for Foods, 1986). They are published in the following well recommended book: "Microorganisms in foods Vol. 2, Sampling for microbiological analysis: Principles and specific applications". This work was meant as an attempt of a universally applicable sampling strategy. Some of the ideas were taken from FOSTER plan and modified. The "ICMSF Sampling Book" also works with risk categories, here called cases. The 15 possible cases deriving from a combination of five risk classes with three of treatment are shown in table 3.

**Table 3: Suggested sampling plans for combinations of degrees of health hazards and conditions of use (ICMSF 1986).**

| Degree of concern relative to utility and health hazard | Conditions in which food is expected to be handled and consumed after sampling in the usual course of events | | |
|---|---|---|---|
| | Conditions reduce degree of concern | Conditions cause no change in concern | Conditions may increase concern |
| No direct health hazard, utility e.g. shelf life and spoilage | Increased shelf life<br>Case 1<br>3-class, n = 5, c = 3 | No change<br>Case 2<br>3-class, n = 5, c=2 | Reduced shelf-life<br>Case 3<br>3-class, n = 5, c = 1 |
| Health hazard low, indirect (indicator) | Reduced hazard<br>Case 4<br>3-class, n = 5, c = 3 | No change<br>Case 5<br>3-class, n = 5, c = 2 | Increase hazard<br>Case 6<br>3-class, n = 5, c= 1 |
| Moderate, direct, limited spread | Case 7<br>3-class, n= 5, c= 2 | Case 8<br>3-class n = 5, c = 1 | Case 9<br>3-class, n = 10, c = 1 |
| Moderate, direct potentially extensive spread | Case 10<br>2-class, n = 5, c = 0 | Case 11<br>2-class, n = 10, c = 0 | Case 12<br>2-class, n = 20, c = 0 |
| *Severe direct | Case 13<br>2-class, n = 15 c = 0 | Case 14<br>2-class, n = 30,c= 0 | Case 15<br>2-class, n = 60, c = 0 |

* Includes *Salmonella*

### 2.6.8 Training of personnel

The competent official authority should, in collaboration with professional organisations and managers as appropriate, arrange for training courses, not only for the employees but also for inspectors in meat processing establishment. The emphasis should be laid on the importance of the hygienic handling of meat and especially on the critical control points of these processes during *Salmonella* contamination, growth and cross-contamination may chiefly occur (WHO, 1994).

Education of consumers and of food handlers in food service establishment on the safe handling and cooking of potentially hazardous meats and other raw ingredients constitutes an important fact of a comprehensive control program. Current national statistics on causes of foodborne outbreaks involving basic food preparation steps such as adequate refrigeration and thorough cooking of foods indicate that the educational needs have yet to be satisfied (Stoll, 1993).

# 3. MATERIALS AND METHODS

## 3.1 Description of study area and population

### 3.1.1 Study area

The study was carried out in Addis Ababa, at the main abattoir and 22 selected supermarkets in the city. The cattle population is estimated at 58.568 (1999 estimates). Addis Ababa has cool climate with an average rainfall of 1800 mm and is situated 2500 m above sea level. The main rain season extends from June to September with an average rainfall of 866 mm (84 % of rain in the year). Rainfall and temperature data from the National Meteorological Services Agency (NMCA 1999) indicate also a short rainy season from March to May. The annual average temperatures are maximum 26 °C, minimum 11 °C with an overall average of 18.7 °C. Highest temperatures are reached in May. Day length is fairly constant throughout the year, at 12-13 hours, with about 6 hours of sunshine during rainy months and 8-10 hours for the rest of the year (NMCA, 1999).

### 3.1.2 Study population

The study population included random sampling of 235 cattle, with the total number of 564 specimen which included 47 pooled samples, containing five single samples of faeces and mesenteric lymphnodes, 235 samples each of abdominal (obliquus and transversus) and diaphragmatic muscle. The sampling design is illustrated in table 4. This study was later followed by sampling of 300 stool samples from the slaughterhouse personnel. From 22 supermarkets, 5 different minced beef samples were collected, once in a week for three weeks, resulting in an overall of 330 samples.

Table 4: Types of specimen sampled in the cross-sectional cattle study

| Number of Animals Examined Per week | Type of Specimen and Number | | | |
|---|---|---|---|---|
| | Faeces pooled | Mesenteric lymphnodes pooled | Diaphragmatic Muscle | Abdomen Muscle |
| 25 | 5 | 5 | 25 | 25 |
| 25 | 5 | 5 | 25 | 25 |
| 20 | 5 | 5 | 20 | 20 |
| 20 | 5 | 5 | 20 | 20 |
| 20 | 5 | 5 | 20 | 20 |
| 25 | 5 | 5 | 25 | 25 |
| 25 | 5 | 5 | 25 | 25 |
| 25 | 5 | 5 | 25 | 25 |
| 25 | 5 | 5 | 25 | 25 |
| 25 | 5 | 5 | 25 | 25 |
| **Total number of specimens:** | | | | |
| **264** | **47** | **47** | **235** | **235** |

The present study is a cross-sectional study and involved determination of the prevalence and distribution of *Salmonella* in faeces, lymphnodes and two different beef cuts from the abattoir and minced beef from supermarkets. The program included weekly visits on fixed days at the abattoir and supermarkets. At the abattoir an average of 600 cattle, 150 pigs, 500 sheep and goats are slaughtered daily. The study population involved sampling of 20-25 animals weekly. 300 stool samples from the 700 abattoir workers were drawn. Identification and classification of the most common serovars of *Salmonella* in animals and products as well as in humans was also conducted. In order to assess some factors contributing to *Salmonella,* a questionnaire was also administered to abattoir personnel and observations on pre-slaughter conditions, slaughter facilities, post-slaughter handling and status of the supermarkets.

### 3.1.3 Samples investigated

The samples investigated from the abattoir were faeces, mesenteric lymphnodes, diaphragm and abdominal muscles from slaughtered cattle. Minced beef samples destined for consumption were bought from the supermarkets in Addis Ababa. Stool samples from the abattoir workers were collected in collaboration with the medical personnel in the abattoir clinic.

All samples were taken under aseptic conditions and transported in a cool box to the Microbiology Laboratory of Faculty of Veterinary Medicine, immediately after collection and cooled at 2-4 $^{O}$C. All samples were labelled accordingly and accompanied with the necessary identifying information. This included the date of sampling, type of specimen and its source.

## 3.2 Culture Method

For the culture method, the technique recommended by the International Organization for Standardization (ISO 6579, 1998), was used (Figure1).

**Figure 1: Diagram of procedure: ISO 6579 (1998)**

Test portion, 25 g + Pre-enrichment medium, 225 ml
incubation at 37 °C for 18 h to 20 h

↓

0.1 ml of culture ← Selective enrichment → 1 ml of culture

10 ml of R.V. broth
(magnesium chloride / malachite green/
soya peptone broth)
incubation at 42 °C for 24 h
(and in necessary for further 24 h)

10 ml of Selenite Cystine broth
incubation at 37 °C for 24 h
(and if necessary for further 24 h)

↘ ↙

Plating out on selective
media in Petri dishes

↙ ↘

1st solid selective plating-out medium (BPLS) ↘

2nd solid selective plating-out medium (MacConkey) ↙

Incubation at 37 °C
for 20 h to 24 h (and for a
further 18 h to 24 h if necessary)

↓

At least five characteristic colonies
(from each plate)

↓

Incubation on nutrient agar

↓

Incubation at 37 ° C for 18 h to 24 h

↘ ↙

Biochemical confirmation Serological confirmation

↘ ↙

Interpretation of results

## 3.3 Sampling Procedures and Processing

- On the first level pooled samples of faeces (containing five single samples) were collected from slaughtered cattle at the Addis Ababa abattoir from 235 animals. 2-3 g samples of faeces were added to Rappaport Vassiliadis (RV) broth for one day.The following day 0.1 ml was transferred to the next RV, after 1 and 2 days streaking on two different plates of BPLS and MacConkey.

- On the second level pooled samples of mesenteric lymphnodes (containing five single samples) were collected from the same number of animals. 25 g were addded to 225 ml of peptone water for one day and on the next day transferred to RV and SC, on the third day streaking onto selective agar medium (BPLS and MacConkey).

- On the third level collection of single beef cuts from diaphragama and abdominal muscles separately weighing 25 g from 235 animals. They were treated as in the second level.

- Additionally, collection of stool samples from 300 abattoir workers, weighing 2-3 g and treatment as in first level.

- On the fourth level, collection of 300 minced beef samples from 22 supermarkets, weighing 25 g. Treatment as in second level.

## 3.4 Culture procedures

### 3.4.1 Pre-enrichment

Diaphragm and abdominal (M obliquus and M. transversus) muscles of 25 g, mesenteric lymphnodes as pooled 25-50 g, raw minced meat 25 g, all separately were mixed using a stomacher in 225 ml buffered peptone water (BPW) and incubated at 37 °C for 16-20 hours for pre-enrichment. Cattle and human faeces samples do not need any pre-enrichment.

### 3.4.2 Enrichment in selective liquid media

After pre-enrichment, 0.1 ml of the separate culture was transferred to 10 ml of Rappaport-Vassiliadis broth and 1 ml to 10 ml of selenite cystine broth to be incubated for 24 hrs at 42 °C and 37 °C respectively. Cattle faeces and human stool were enriched in 10 ml Rappaport-Vassiliadis broth for one day and transferred to the next RV on the next day.

### 3.4.3 Plating out and recognition

From the cultures obtained in 3.4.2. each sample was plated out on BPLS- and MacConkey agar medium and incubated at 37 °C for 24 hours. The plates were examined for the growth of *Salmonella* colonies. If growth is slight or if no typical colonies of *Salmonella* are present on BPLS and MacConkey agar, they were reincubated at 37 °C for further 18-24 hours. About five suspected colonies were selected from each plate and streaked on nutrient agar. After streaking of the suspected colonies, they were incubated at 37 °C for 24 hours. They was re-examined for the presence of *Salmonella*. The suspected colonies were subjected to confirmation using biochemical tests.

## 3.5 Biochemical and Serological tests

The isolates were biochemically differentiated from other Gram–negative genera using reaction on TSI agar slopes, Lysine decarboxylase and Simon's citrate agar slopes. These were performed in order to increase the likelihood of obtaining suspected *Salmonella* colonies for serological confirmation. Apart from TSI agar slopes, lysine decarboxylase and Simon's citrate agar slopes, the biochemical tests were carried out using " Enterotube II" (Roche-Diagnostic, Basel) as described by the manufacturer. For this purpose only obtained pure colonies were used. The tubes were incubated overnight at 37 °C, after inoculation. The reactions were recorded on the interpretation pad. They were considered negative only if the compartments remain unchanged with the exception of indole and Vosges-Proskauer. These two were tested by adding their respective reagents. The tubes were read by writing down the numbers for each positive reaction on the interpretation pad in comparison with a reference pattern supplied with the product from the manufacturer. The numbers i.e. the values for each positive reaction were added and then a five digit number (ID value) was obtained, referring to the CCIS (Computer Coding and Identification System) for "Enterotube II" (Hoffman-La Roche and Co. AG, Switzerland).

Suspected *Salmonella* isolates were tested further using anti-*Salmonella* sera I and II as well as with group specific anti-*Salmonella* sera following the recommendations of the manufacturer (Sifin, Berlin). From isolates reacting to groups B, C, D or E, a single colony was transferred to nutrient agar and incubated for 24 hours and at 37 °C. The isolates were then transported maintaining the cold chain to the Institut für Lebensmittelhygiene for validation and finally to the BgVV (Bundesinsitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Berlin) for final confirmation. Originally in Ethiopia 118 *Salmonella* strains were isolated. After storing, transport and recultivation, finally 98 *Salmonella* strains were serotyped.

## 3.6 Serotyping at BgVV, Berlin

For serotyping, isolates were first plated on Gassner Agar and incubated overnight at 37 °C. A small amount of one single colony was mixed with rabbit-anti sera against "O"-antigens to check for agglutination. Afterwards, from the same colony a small amount was transferred to semi-solid agar plate and incubated overnight. Testing was done in the same way, looking for the second H-phase of *Salmonella*. Results of the first day were confirmed by repeated testing.

All anti-sera used were prepared by the Reference Laboratory by their own. First pools of sera were used for grouping, afterwards all individual sera included in a group which showed positive agglutination were tested individually. Finally, using the antigen formula in the Kauffmann-White scheme, the serovar was determined.

## 3.7 Testing for antibiogram in BgVV, Berlin

Seventeen antibiotics were tested against the strains detected. These drugs were the following (tab. 3): Amikacin (AK 30 μg), Ampicillin (AMP 10 μg), Cefuraxolin (CXM 30 μg), Chloramphenicol (C 30 μg), Colistin (CT 10 μg), Enrofloxacin (ENR 5 μg), Furazolidon (FR 100 μg), Gentamycin (CN 10 μg), Kanamycin (K 30 μg), Nalidixin acid

(Na 30 µg), Neomycin (N 10 µg), Polymyxin B (PB 300 IU), Streptomycin (S 25 µg), Sulphamethoxazol/Trimethoprim (SXT 25 µg), Sulphonamide (SU 300 µg), Tetracycline (TE 300 µg) and Trimethoprim (W 25 µg). Testing was done following the procedure described by Arbeitskreis für Veterinärmedizinische Infektionsdiagnostik (AVID 1996). Briefly, an agar diffusion test was carried out using Mueller-Hinton agar. Each strain was cultivated first in L-broth overnight at 37 °C. Afterwards culture was diluted adequately and plated on Mueller-Hinton agar to enable growth of non-confluenting colonies on the plate. Commercially (OXOID) available platelets containing standardized amounts of antimicrobials were placed on the agar plates inoculated with the strain to be tested. Interpretation was done following incubation overnight at 37 °C by reading the zone of inhibition of growth. The diameter of the zone surrounding each antimicrobial disk with no bacterial growth was determined criteria for scoring test strains as sensitive, intermediate or resistant were used according to DIN 58940 (Deutsches Institut für Normung e.V., 1989) interpretative standards.

**Table 5 : Antibiotic disks used, their symbols and concentration per disk**

| Antibiotic | Symbol | Concentration(µg/disk) |
|---|---|---|
| Amikacin | AK | 30 |
| Ampicillin | AMP | 10 |
| Cefuraxolin | CXM | 30 |
| Chloamphenicol | C | 30 |
| Colistin | CT | 10 |
| Enrofloxacin | ENR | 5 |
| Furazolidon | FR | 100 |
| Gentamycin | CN | 10 |
| Kanamycin | K | 30 |
| Nalidixic acid | NA | 30 |
| Neomycin | N | 10 |
| Polymyxin | PB | 300 (IU) |
| Streptomycin | S | 25 |
| Sulphametoxazole-trimethoprim | SXT | 25 |
| Sulphonamide | SU | 300 |
| Tetracycline | TE | 30 |
| Trimethoprim | W | 2,5 |

## 3.8 Data Analysis

All the data collected from samples of the abattoir cattle (pooled faeces and mesenteric lymphnodes, muscles from diaphragm and abdominal), abattoir workers and minced beef from supermarkets were stored and analyzed using the software Microsoft Excel. Analysis of data on the prevalence and distribution of *Salmonella* are presented in the form of tables and figures.

To compare the pool prevalence for faeces and mesenteric lymphnodes with the *Salmonella* prevalence of individual muscle samples of abdominal and diaphragm it was calculated using formula shown below. With the help of this formula, the individual-level prevalence is estimated on the basis of pool samples.

$$\textbf{Calculated prevalence : } P = 1 - (1 - \text{pool prevalence})^{1/n}$$

P = prevalence of single samples

n = pool size; in this case 5.

The second formula for the created pool from individual single samples that based on one or more *Salmonella* was considered as a positive pooled sample (Cowling et al., 1999).

$$\textbf{Calculated } Pp = 1 - (1 - P)^5$$

Pp = pool prevalence

P = single prevalence (in this case N / 235,

"N" is the number of *Salmonella* positive cases)

Graphs were drawn using MS Excel to illustrate the prevalence and distribution of *Salmonella* from the samples. The statgraphics plus 2.1 statistical software (Magnugistics, Inc., Rockville, Ma., USA) was used for the Chi-square test to see whether salmonellosis of the abattoir cattle was associated with animal condition, mode of transport, source of minced beef from supermarkets and storage conditions. Calculation was done according to Martin et al., (1987). Antimicrobial results were analysed based on the description by AVID (1996).

# 4. RESULTS

This was a cross sectional study whose objective was to estimate the prevalence and distribution of *Salmonella* from 235 abattoir cattle, selected as random samples.. The sources analyzed were pooled cattle faeces, pooled mesenteric lymphnodes, single muscles from the diaphragm as well as from the abdominal region. Additional minced beef from supermarkets destined for human consumption and additinally stool samples from the abattoir personnel were included in the survey. The isolated Salmonella–strains were serotyped and resistance against antibiotics determined.

*Salmonella* isolates from 47 pooled faeces samples were 5 (10.6 %), from 47 pooled mesenteric lymphnodes 9 (19.6 %), from 235 single samples each of the abdominal (M. obliquus and M. transversus) muscle 23 (9.8 %) and of the diaphragmatic muscle 28 ( 11.9 %). In 300 stool samples from the abattoir personnel 18 isolates (6.0 %) *Salmonella* have been detected. Minced beef from the 22 supermarkets with an overall of 330 samples contained *Salmonella* in 26 cases ( 7.9 %), as shown in table 6.

A questionnaire was administered to get more information about the abattoir's pre-slaughter conditions, slaughter facilities, post-slaughter handling and disease status, furthermore the association of salmonellosis in the abattoir cattle with the condition of animals and mode of transport, and finally about the prevalence of *Salmonella* in minced beef from supermarket, with association to storage conditions.

In order to establish a link between serovars and their potential to public health hazard, the most common serovars of *Salmonella* from the abattoir cattle and personnel on the one hand and minced beef from supermarkets on the other were defined. Furthermore, the antibiotic susceptibility of *Salmonella* isolates to commonly used antimicrobial agents was tested due to the significance to public health from the viewpoint of food hygiene and the therapy of salmonellosis.

In the study 98 Salmonella strains were serotyped and identified as 27 *S.* Anatum 53 *S.* Dublin, 5 *S.* Meleagridis, 1 *S.* Muenchen, 9 *S.* Saintpaul and 3 *S.* rough form. All 98 strains reacted sensitive against the seventeen antimicrobial agents used.

**Table 6: Frequency of *Salmonella* isolates by source**

| Source | Number examined | Number of isolates | Positive samples (%) |
|---|---|---|---|
| **Abattoir:** Cattle | | | |
| **Pooled faeces** | **47** | **5** | **10.6** |
| **Pooled. Lnn. mes.** | **47** | **9** | **19.2** |
| Estimated faeces | 235 | 5 | 2.1 |
| Estimated Lnn. mes. | 235 | 10 | 4.3 |
| **Ab. Muscle** | **235** | **23** | **9.8** |
| **Dia. Muscle** | **235** | **28** | **11.9** |
| C. ab. Muscle | 47 | 19 | 40.4 |
| C. dia. Muscle | 47 | 22 | 46.8 |
| **Abattoir :** Personnel | | | |
| **Human stool** | **300** | **18** | **6.0** |
| **Supermarket** | | | |
| **Minced beef** | **330** | **26** | **7.9** |

Bold lines contains the original data

Lnn. mes. = mesenteric lymphnodes

Ab. = Abdominal

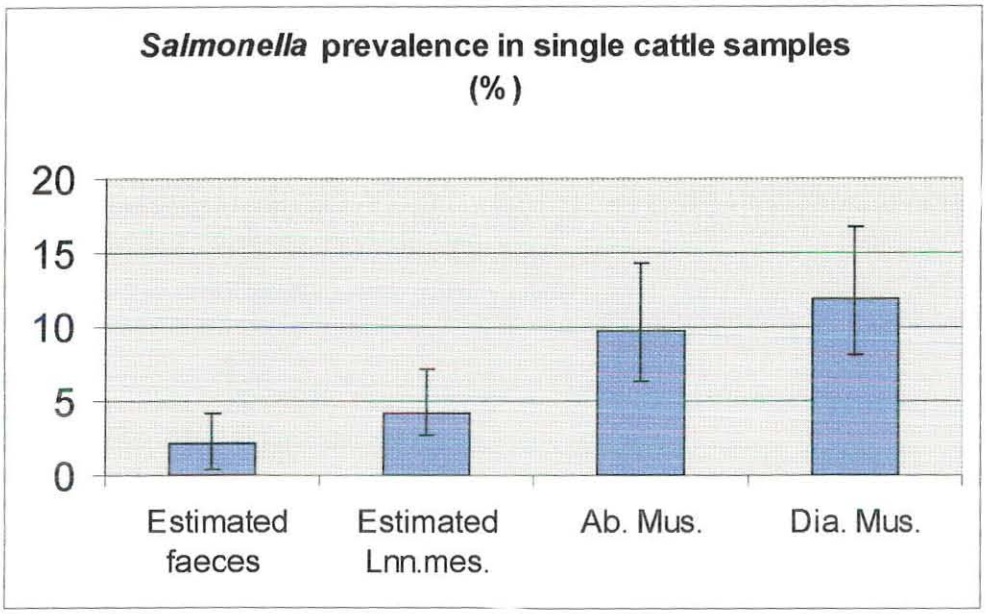
Dia. = Diaphragm

Table 6 shows the frequency of *Salmonella* isolation by source. 235 abattoir cattle were sampled. From each cattle single samples of mesenteric lymphnodes and faeces are taken. For both matrices 5 single samples have been combined to a pooled sample, leading to 47 pool samples for each matrix. Further 235 single samples of diaphragmatic muscle and 235 single samples of abdominal muscles are separately drawn. Additionally, the study included 300 stool samples from the abattoir personnel and 330 minced beef samples from the supermarkets. The following numbers of *Salmonella* were isolated: pooled faeces 5/47 (10.6 %), pooled mesenteric lymphnodes 9/47 (19.6 %), abdominal (obliquus and transversus) 23/235 (9.8 %) and diaphragmatic muscle 28/235 (11.9 %), human stool 18/300 (6.0 %) and minced beef 26/330 (7.9 %). The estimation of the single samples based on pooled ones was calculated with the help of the formula given on page 28 led to the following results: for faeces 5/47 x 5 (2.1 %) and for lymphnodes 10/47 x 5 (4.3 %). The conversion of individual samples into created pool samples was also done using the formula given on page 28.

The computation for the abdominal and diaphragm muscles is as follows: 19/47 (40.6 %) and 22/47 (46.8 %) respectively.

## 4.1 Detailed analysis of the cattle data

**Fig 2: *Salmonella* prevalence in single cattle samples**




**Lnn. mes. = mesenteric lymphnodes**

**Dia. Mus. = Diaphragm Muscle**

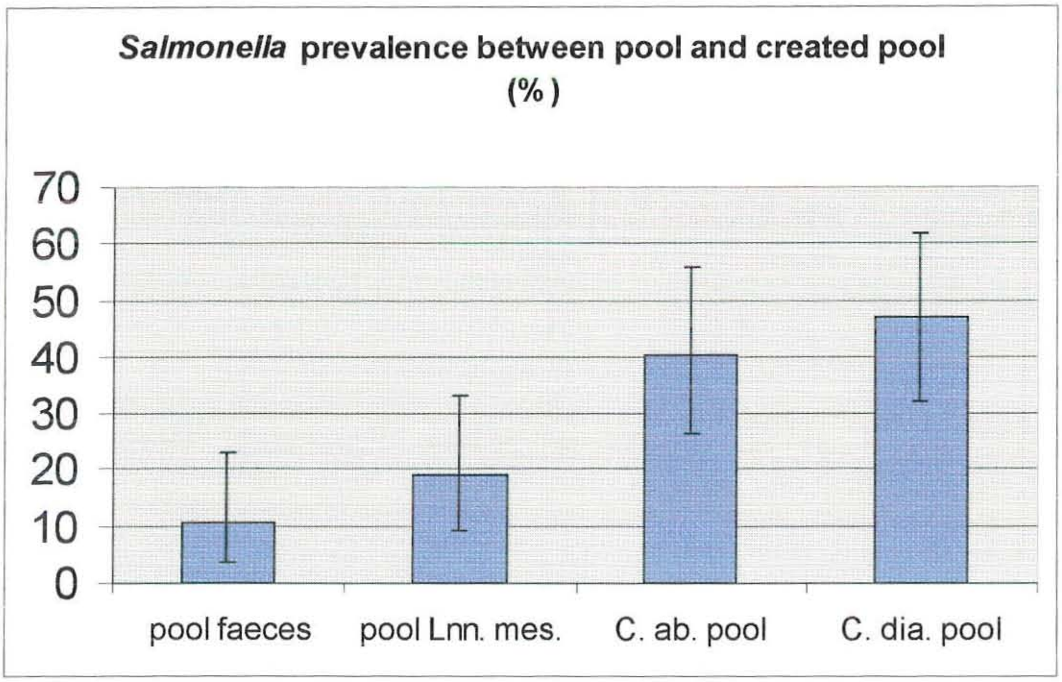
**Ab. Mus. = Abdominal Muscle**

**Calculated prevalence: $P = 1 - (1 - \text{Pool Prevalence})^{1/5}$**

The above formula was used to calculate the individual prevalence for faeces and mesenteric lymphnodes from pooled samples. This is done in order to make it comparable with the prevalence for individual samples of abdominal and diaphragm muscles. The mathematical procedure for estimation of individual-level prevalence based on pool samples was reviewed by Cowling et al. (1999). The estimated prevalence for single samples was 2.1 % in faeces and 4.25 % in mesenteric lymphnodes respectively.

Fig. 2 illustrates the overlapping of the exact binomial confidence intervals (CI) indicating no significant difference between the four groups. The prevalence of *Salmonella* estimated as individual level prevalence based on pool samples, where isolates were 5 ( 2.1 %), CI = 0.36 (lower limit = LL) to 4.22 (upper limit = UL) and mesenteric lymphnodes 10 ( 4.26 %), CI = 2.68 (LL) to 7.15 (UL). For 235 single samples of beef cuts the corresponding data are: abdominal muscles 23 ( 9.8 %), CI = 6.3 (LL) to 14.3 (UL) and diaphragma muscle 28 (11.9%) CI = 8.11 (L.L) to 16.8 (UL).

**Fig. 3: *Salmonella* prevalence of the pooled vs. created pool (%)**




Lnn. mes. = Mesenteric lymphnodes
C. ab. = Created abdominal pool
C. dia. = Created diaphragm pool

**Created pooled data (5 samples were considered as one pool for analysis)**

To compare the cattle data on a pool level the results of the muscles analysis were transformed to "created pooled data". Created pools means summing up the results of five cows (following row of numbers). Every sample of five with one or more *Salmonella* positive was considered as a positive pooled sample. At the end there have been 47 created pools (containing 5 single samples). In detail there were 24 pools without *Salmonella*, those comprising Salmonella positive were 12 pools with 1, 5 pools with 2, 4 pools with 3, 1 pool with 4 and 1 pool with 5 respectively. The prevalence of created pools was calculated as P pool = 23/47 = 0.489.

Figure 3 shows that the exact binomial confidence intervals are mainly overlapping and there is no significant difference between pooled and created pool with the exception of pooled faeces samples. The calculated values are (real pool) faeces 5 (10.6 %), CI = 3.5 (LL) to 23.1 (UL), mesenteric lymphnodes real pool 9 (19.2 %), CI = 9.14 (LL) to 33.3 (UL), diaphragm muscle created pool 19 (40.3 %,) CI = 26.4 (L.L) to 55.7 (UL) and abdominal muscle created pool 22 (46.8 %) CI = 32.1 (LL) to61.9 (UL).

**Fig. 4: The observed vs. the expected distribution of the number of positive samples in created pools (size of pool n = 5) abdominal muscle and diaphragm muscle**

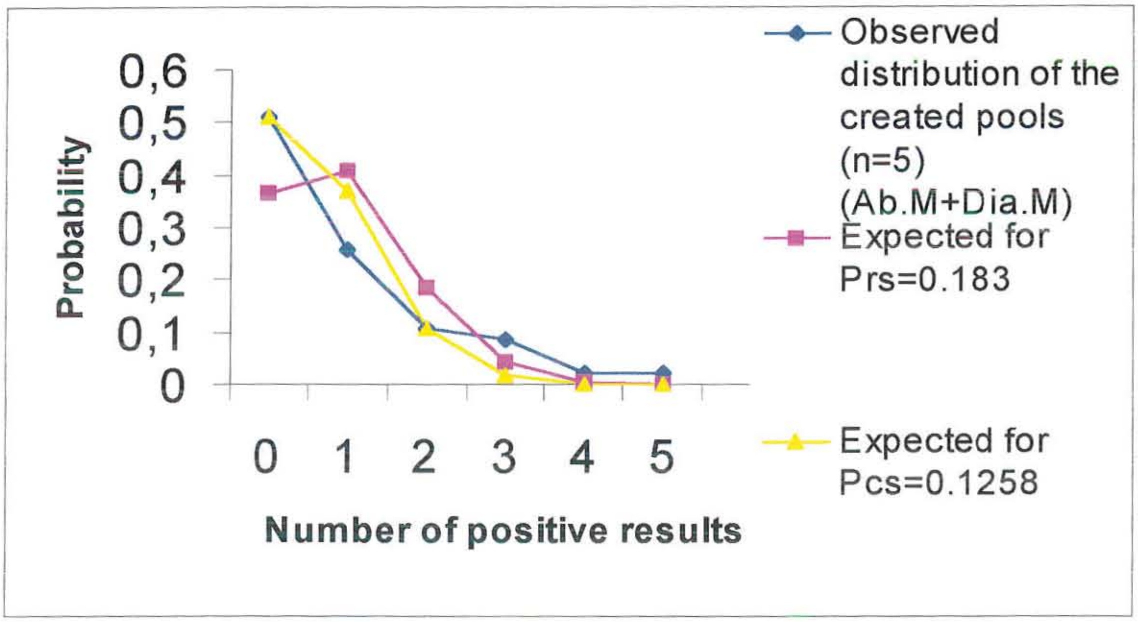



Fig. 4 illustrates the observed distribution of the created pools with 0, 1, 2, 3, 4 and 5 positive single samples. The good correspondence of the two values for probability of real single (Prs) and probability of single estimated from created pool (Psc) shows that the results seem to be in agreement with the mode of the binomial distribution. The condition is allowed, that the cattle samples are drawn randomly and no bias exists. With the help of the formula

**Calculated prevalence: $P = 1 - (1 - \text{Pool Prevalence})^{1/5}$**

it was possible to calculate the probability Pcs for a single to be *Salmonella* positive on the basis of the created pools. The calculated value amounts Pcs = 0.125 if binomial distribution was accepted. In the next step the probability of 0, 1, 2, 3, 4 and 5 positive results in the pool of 5 samples was calculated and drawn in Fig. 4 as "expected for Pcs = 0.126". The third distribution curve is calculated with help of the same principles but using the real prevalence Prs = 43/235 (0.183.)

### 4.2 Abattoir personnel stool data

Table 7 illustrates the 300 human stool samples examined, the *Salmonella* prevalence was 18 ( 6.0 %) and CI = 3.6 (LL) to 9.3. (UL).

**Table 7: Confidence interval of *Salmonella* prevalence in abattoir personnel stool**

| Source | No. examined | No. Positive | Prevalence (%) | UL | LL |
|---|---|---|---|---|---|
| Human stool | 300 | 18 | 6.0 | 9.3 | 3.6 |

### 4.3 Detailed analysis of the minced beef data

Table 8 illustrates the *Salmonella* distribution detected in the supermarket samples in Addis Ababa. Fifteen samples of minced beef were collected from each supermarket, therefore from 22 supermarkets a total of 330 specimens were sampled of which 26 (7.9 %) contained *Salmonella*. The range was 0 up to 3 isolates per 15 samples. Table 8 shows the abattoir source of minced beef, too.

Table 8: *Salmonella* isolation in minced beef from supermarkets

| Supermarket No. | Source | No. examined | No. of samples positive for *Salmonella* |
|---|---|---|---|
| 1 | 2 | 15 | 1 |
| 2 | 1 | 15 | 0 |
| 3 | 3 | 15 | 1 |
| 4 | 1 | 15 | 1 |
| 5 | 2 | 15 | 1 |
| 6 | 2 | 15 | 1 |
| 7 | 1 | 15 | 1 |
| 8 | 2 | 15 | 2 |
| 9 | 1 | 15 | 5 |
| 10 | 2 | 15 | 3 |
| 11 | 1 | 15 | 0 |
| 12 | 1 | 15 | 0 |
| 13 | 2 | 15 | 0 |
| 14 | 1 | 15 | 1 |
| 15 | 1 | 15 | 1 |
| 16 | 1 | 15 | 0 |
| 17 | 1 | 15 | 1 |
| 18 | 3 | 15 | 2 |
| 19 | 1 | 15 | 2 |
| 20 | 2 | 15 | 1 |
| 21 | 1 | 15 | 2 |
| 22 | 2 | 15 | 0 |
| **Total number of specimen** | | **330** | **26 (7.9 %)** |

1 = Addis Ababa abattoir; 2 = Kaliti abattoir; 3 = Kaliti abattoir

Fig 5: *Salmonella* prevalence in minced beef by abattoir sources

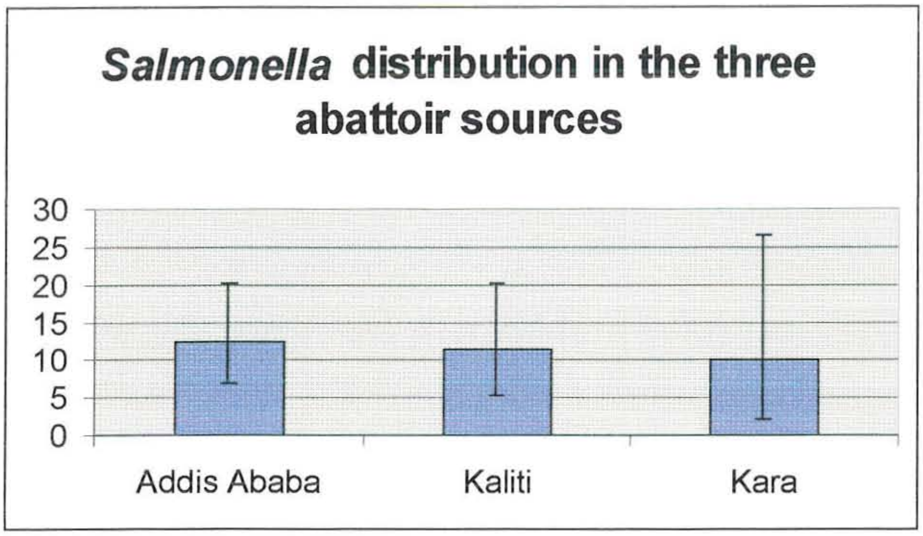



Fig. 5 illustrates the overlapping of the binomial confidence interval between abattoir sources at 95 % binomial confidence interval. This shows that there is no significant difference between the abattoir sources. For the three abattoir sources from the supermarkets sampled there is given: the sample size, the number of *Salmonella* isolates and the *Salmonella* prevalence with the binomial confidence intervals respectively: Addis Ababa abattoir 105, 13 ( 12.4 %), CI = 6.8 (LL) to 20.2 (UL); Kaliti abattoir 80, 9 (11.3 %), CI = 5.3 (LL) to 20.3 (UL); Kara abattoir 30, 3 (10.0 %) CI = 2.1 (LL) to 26.5 (UL).

**Table 9: *Salmonella* isolations associated with animal condition**

| **Animal condition** | *Salmonella* (+) | *Salmonella* (-) | **total** | **Positive (%)** |
|---|---|---|---|---|
| Poor (0) | 11 | 1 | 12 | 91.7 |
| Average (1) | 18 | 83 | 101 | 21.7 |
| Good (2) | 14 | 97 | 111 | 12.6 |
| Excellent (3) | 0 | 11 | 11 | 0 |
| **Total** | **43** | **192** | **235** | |

The Chi–square test was used to see if there was any association of salmonellosis in animals and their condition. Of 235 animals sampled, 192 were *Salmonella* negative and 43 positive table 9. The frequency of salmonellosis in animals according to their condition were: poor status: 91.7 %, average: 21.7 % good: 12.6 % and in excellent (healthy animals): 0 %.

The table also shows that there was significant difference ($X^2 = 48.09$, d.f = 3, $P < 0.05$) in *Salmonella* prevalence rate and animal condition.

**Table 10: *Salmonella* isolations associated with the mode of transport**

| mode of transport | *Salmonella* (+) | *Salmonella* (-) | Total |
|---|---|---|---|
| Foot | 13 | 61 | 74 |
| Rail | 18 | 71 | 89 |
| Road | 12 | 60 | 72 |
| **Total** | **43** | **192** | **235** |

Table 10 shows the mode of transport of the animals to the abattoir and *Salmonella* isolation rate. It was done by hoof, rail or road. Of the 235 animals sampled, 192 were negative and 43 were *Salmonella* positive. The Chi–Square test was used to see if there was any association in the abattoir between disease status and mode of transport. Table 10 shows that there was no significant difference ($X^2 = 0.38$, d.f = 2, $P > 0.05$) in *Salmonella* prevalence rate and mode of transport.

**Table 11: *Salmonella* isolations in minced beef from the supermarkets associated with storage**

| storage | *Salmonella* (+) | *Salmonella* (-) | Total |
|---|---|---|---|
| cold room (0) | 3 | 1 | 4 |
| refrigerator (1) | 12 | 3 | 15 |
| not cooled (2) | 1 | 2 | 3 |
| Total | 16 | 6 | 22 |

Table 11 illustrates the *Salmonella* detection in minced beef by storing in the coldroom, refrigerator and non cooled facilities. *Salmonella* isolates were 16 positive and 6 negative in an overall of 22 samples. The Chi–Square test was used to see if the prevalence of *Salmonella* in minced beef of supermarkets was linked with storage temperature. The table shows that there was no significant difference ($X^2 = 2.76$, d.f = 2, $P > 0.05$) in *Salmonella* prevalence rate and storage.

## 4.4 Results of serotyping

Table 12 illustrates the results of serotyping of 72 *Salmonella* strains isolated from the abattoir cattle and personnel.

**Table 12: *Salmonella* serotypes isolated from abattoir samples**

| Source | No. of *Salmonella* isolates | | | | |
|---|---|---|---|---|---|
| | SA | SD | SME | SMU | SR |
| **Abattoir**: Cattle | | | | | |
| Faeces pooled | | 4 | | 1 | |
| Lnn. mes. | | | | | |
| Pooled | 3 | 2 | | | |
| abdominal muscle | 3 | 18 | | | |
| diaphragmatic muscle | 2 | 21 | | | |
| **Abattoir:** personnel | 7 | 4 | 5 | | 2 |
| **Total** 72 | **15** | **49** | **5** | **1** | **2** |

SA = *Salmonella* Anatum

SD = *Salmonella* Dublin

SME = *Salmonella* Meleagridis

SMU = *Salmonella* Muenchen

SRF = *Salmonella* rough form

From the pooled samples of faeces (containing 5 single samples) 4 *S.* Dublin and 1 *S.* Muenchen, of the pooled samples of mesenteric lymphnodes (containing 5 single samples) 3 *S.* Anatum and 2 *S.* Dublin have been found. Similar to the cattle faeces, *S.* Dublin dominated in the beef cuts single samples. In single samples of abdominal muscles 18 *S.* Dublin and 3 *S.* Anatum and in diaphragm muscle samples 21 *S.* Dublin and 2 *S.* Anatum occurred. The 18 *Salmonella* isolates from the abattoir personnel consisted of 7 *S.* Anatum, 4 *S.* Dublin, 5 *S.* Meleagridis and 2 *Salmonella* rough forms. In total the distribution of 72 *Salmonella* serovars runs as follows: 15 *S.* Anatum, 49 *S.* Dublin, 5 *S.* Meleagridis, 1 *S.* Muenchen and 2 *S.* rough form, respectively.

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| Pooled | 3 | 2 | | | |
| abdominal muscle | 3 | 18 | | | |
| diaphragmatic muscle | 2 | 21 | | | |
| **Abattoir:** personnel | 7 | 4 | 5 | | 2 |
| **Total 72** | **15** | **49** | **5** | **1** | **2** |

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Table 13 shows the frequency of Salmonella serotypes in minced beef from the three abattoir sources.

**Table 13: The frequency of Salmonella serotypes in minced beef from supermarkets in three abattoir sources**

| Supermarket No. | Source | No. of *Salmonella* isolates | | | |
|---|---|---|---|---|---|
| | | SA | SD | SSp | SR |
| 1 | 2 | | 1 | | |
| 2 | 1 | | | | |
| 3 | 3 | | | 1 | |
| 4 | 1 | 1 | | | |
| 5 | 2 | | | 1 | |
| 6 | 2 | | | 1 | |
| 7 | 1 | 1 | | | |
| 8 | 2 | | | 2 | |
| 9 | 1 | 3 | 1 | | 1 |
| 10 | 2 | 2 | | 1 | |
| 11 | 1 | | | | |
| 12 | 1 | | | | |
| 13 | 2 | | | | |
| 14 | 1 | | 1 | | |
| 15 | 1 | 1 | | | |
| 16 | 1 | | | | |
| 17 | 1 | 1 | | | |
| 18 | 3 | | | 2 | |
| 19 | 1 | 2 | | | |
| 20 | 2 | | | 1 | |
| 21 | 1 | 1 | 1 | | |
| 22 | 2 | | | | |
| **Total 26** | | **12** | **4** | **9** | **1** |

SA = *Salmonella* Anatum — 1 = Addis Ababa abattoir

SD = *Salmonella* Dublin — 2 = Kaliti abattoir

SSp = *Salmonella* Saintpaul — 3 = Kara abattoir

SRF = *Salmonella* rough form

Of the 26 *Salmonella* strains are 12 *S.* Anatum, 4 *S.* Dublin, 9 *S.* Saintpaul and 1 *S.* rough form. Arranged for the abattoir sources of the *Salmonella* 3 *Salmonella* serotypes (2 *S.* Anatum, 1 *S.* Dublin and 6 *S.* Saintpaul) originated from Kaliti abattoir. From Kara abattoir all 3 strains belonged to *S.* Saintpaul. In the minced beef from Addis Ababa 10 *S.* Anatum, 3 *S.* Dublin and 1 *S.* rough form were found. In all the samples of Addis Ababa abattoir (faeces, mesenteric lymphnodes, beef cuts and minced meat) no *S.* Saintpaul was detected.

## 4.5 Antimicrobial test results

All the 98 *Salmonella* strains, namely *S.* Anatum, *S.* Dublin, *S.* Meleagridis, *S.* Muenchen, *S.* Saintpaul and *S.* rough form isolated from the abattoir cattle samples, personnel and minced beef from supermarkets were tested in resistance against the 17 antibimicrobials already shown in the previous table 3. All the *Salmonella* strains were sensitive to the 17 drugs.

## 4.6 Questionnaire survey

In order to assess some factors contributing to *Salmonella* occurrence, a questionnaire was administered to the abattoir personnel and observations on pre-slaughter conditions, slaughter facilities, post-slaughter handling and disease status. Additionally, the conditions, storage facilities and sources of meat to the supermarkets were registered.

### 4.6.1 The pre-slaughter conditions

The source of cattle was from different parts of the country. It was transported by vehicle, rail and foot. The noticed common cattle diseases were tuberculosis (TB), helminthiasis and, in some cases, various metabolic disorders. Animals brought for slaughter were adults aged 5 years or older. Usually, animals were numbered after arrival at the abattoir by giving an abattoir code number. An average of 450-500 animals are slaughtered daily. Disease suspected cattle were additionally screened for TB, blood parasites and Foot and Mouth Disease (FMD). A veterinarian and assistant meat inspectors carry out the ante-mortem inspection.

### 4.6.2 Slaughter Facilities

Birds, rodents and other vermin are attracted by the bones that are exposed outside and do invade the general surrounding of the abattoir. The design and facilities of the slaughter line are both mechanized and manual. The major source of water is chlorinated tap water. A veterinarian and assistant veterinarian meat inspectors carry out the post-mortem inspection. At slaughter the most frequent findings of meat inspection are pathological lesions, pneumonia, emphysema, abscess, calculii, nephritis, pyaemia and TB lesions. The condemned specimens and inedible slaughter offal including intestines and their contents are processed into blood meal, animal concentrates and bone meal for pets, poultry, cattle and other livestock. The disposed waste passes into the main internal drainage and is emptied to the pit-holes and some of it is incinerated.

### 4.6.3 Post-slaughter handling and disease status

The type of laboratory analysis in case of a suspected disease are blood smears and faeces for parasites, Bacillus anthracis, TB and discharges. TB is a reported disease the of the abattoir's personnel. The slaughterhouse storage facilities include cold rooms and refrigerators. The meat is transported in closed trucks and then supplied to private and state owned butcher shops and supermarkets.

## 4.7 Supermarkets

The source of meat from the supermarkets were Addis Ababa, Kaliti and Kara. The mode of transport of meat and meat products are usually closed vehicles. The types of storage facilities are mainly cold rooms and refrigerators. Meat is processed into sausages and minced beef. The disease screening for the personnel is done by public health personnel, in some cases screening for TB, salmonellosis and other zoonotic diseases. Meat and meat products are randomly collected for various laboratory analyses.

# 5. DISCUSSION

This study was planned as a baseline start. The Addis Ababa abattoir was selected as a model to determine the *Salmonella* prevalence from cattle samples, starting with the pooled faeces followed by mesenteric lymphnodes and muscles from the diaphragm as well as from the abdominal region. The prevalence of *Salmonella* was determined up to the end product, that means minced beef sold in Addis Ababa supermarkets. Additionally, the human stool from the abattoir personnel was analyzed for *Salmonella* serotypes.

**Abattoir cross sectional study**

Generally the fact that *Salmonella* occurs at Addis Ababa abattoir is in agreement with studies of other authors on the abattoir. From the current studies *Salmonella* isolates from the diaphragm 28 / 235 (11.9 %) and abdominal 23 / 235 (9.8%) muscles show beef cuts to be the most important source for *Salmonella* in comparison to expected numbers (calculated from pool samples) for individual faeces of cattle for n = 5/47 x 5 (2.1 %) and mesenteric lymphnodes n = 10/47x 5 (3.8 %). Low prevalence in living animal, indicated by low isolation rates of faeces and lymphnodes, leads to the conclusion that the contamination of beef cuts is not due to spreading of *Salmonella* in the living or dying organisms. There must have been severe cross-contamination during the skinning process as a result of bad hygienic conditions during subsequent dressing operations. One probable source could have been from infected abattoir personnel because in 18 ( 6 %) of the 300 stool samples *Salmonella* were isolated. On the other hand in the human faeces *S.* Meleagridis was detected, a serovar which has never been found in cattle (see p. 46). Several reports have stressed the impact of poorly disinfected knives or other slaughtering equipment and bad hygiene among the slaughterhouse personnel on cross contamination (WHO, 1985). In this Addis Ababa study other contamination routes are also probable. Mainly some environmental factors, such as insects, rodents, avian vectors and water could have also been involved in the infection of herds and contamination of processing areas. These vectors have also been demonstrated in abattoirs of other countries to be the potential source of contamination of meat destined for human consumption (Samuel et al., 1980). All the facts mentioned above as causative agents of beef contamination during the slaughter process are also found at the Addis Ababa abattoir.

In previous studies it was reported, that stressful conditions and over-crowding associated with transport of animals from rearing farms to the abattoir induces shedding of *Salmonella* in animals (Samuel et al., 1981). However, from the Addis Ababa studies the Chi-Square test indicates salmonellosis detection in cattle from the abattoir was not correlated with circumstances of animal transport. On the other hand, the results lead to the conclusion that the health status of the cattle is associated with salmonellosis because in the faeces and/or mesenteric lymphnodes of 12 cows classified as "poor conditioned" *Salmonella* was detected in 11 times.

In this survey pool-testing was used for the faeces and mesenteric sample in agreement to Cowling et al. (1999). Collective sample testing can be used to determine herd status (infected or non infected) or, if multiple pools per herd are used, the individual animal prevalence can be estimated with the help of these results as shown in this study.

The consumption of raw meat by the abattoir personnel *Salmonella* was isolated of by stool samples 18 (6 %) of the 300 stool samples may be interpreted as an indication of chain contamination between cattle carcass and people. This is concluded from work carried out in a Nigerian abattoir (Addo and Diallo, 1981). Outbreaks of human salmonellosis as a result of eating raw ground beef have previously been described by Roels et al. (1997). It's advisable to cook meat thoroughly for human consumption to reduce the risks of foodborne illness. Despite some unsanitary practices at the slaughterhouse are detected in Ethiopia, little is known to quantify the risk of contamination of meat with *Salmonella* at that level. For example information is not available on the degree of contamination of the environment with *Salmonella* caused by untreated effluents or the *Salmonella* prevalence in pests like rodents or birds.

**Supermarket cross sectional study**

In the present study the isolation rate of 26/330 (7.9 %) from minced beef of the Addis Ababa supermarkets was less than the rate of 20/50 ( 40 %) samples reported by Molla et al. (1999). The difference between the results of the latter and the present one, though carried out in the same environment, could have been due to improvement of the hygienic standards, seasonal influences or fundamental differences between the shops included in both studies. Especially there was demonstrated a lower *Salmonella* contamination of minced beef in large supermarkets than in local shops. These findings underline a casual relationship between frequent handling and cross contamination of beef products But it is clear that concerning the way from the Addis Ababa slaughterhouse to the consumer, the prevalence of *Salmonella* does not increase during beef transport, production of minced beef and selling it in the supermarkets.

**Questionnaire Survey**

The scope of the questionnaire was gaining more information by an assessment of factors contributing to salmonellosis in the abattoir and in minced beef from supermarkets. Applying this questionnaire, important information about the health hazards of salmonellosis to people and livestock can be obtained. Interviews or other efforts carried out before and after the campaign contribute an important fact of a comprehensive control program against *Salmonella*. As shown by this Addis Ababa study it is necessary to carry out ante-mortem and post-mortem inspection on slaughter animals. Only apparently healthy animals are to be recommended for slaughter in the abattoir, because a much higher risk of salmonellosis can arise from animals of poor condition. Storage did not have any effect on *Salmonella* prevalence of minced beef from the supermarkets, but the sources of beef impact on the isolated serotypes (p. 46).

**Serotypes isolated**

The present study was conducted to detect *Salmonella* serotypes which are common in the Ehtiopian line from stable to table. Taking samples from cattle at the slaughterhouse, beef cuts, abattoir personnel and minced beef should give some information whether there exists a link between the serovars of different steps of pre-and post-harvest meat chain and serovars of potential public health hazard that could be isolated. In this study carried out for the first time on salmonellosis in the Addis Ababa region in abattoir cattle and human personnel, predominant *Salmonella* serotypes were *S.* Dublin followed by *S.* Anatum. Both serovars were mostly isolated from pooled samples of bovine faeces, mesenteric lymphnodes and beef cut samples of the diaphragmatic and abdominal muscle. These results agree with works carried out by various authors concerning different slaughterhouses in Africa and other parts of the world (Miller, 1971; Chambers, 1971; Adesiyun and Oni, 1980; Sharma et al. 1996; FAO/World Bank; 1992; Anonymous, 1997). According to literature *S.* Dublin appears to be almost cattle specific as it is commonly identified in this ruminant. On the other hand *S.* Muenchen which was only isolated from cattle faeces and *S.* Anatum are not host specific.

The *Salmonella* strains from the abattoir personnel comprised *S.* Anatum, *S.* Dublin and *S.* Meleagridis. In this study carried out for the first time on abattoir workers these serotypes are firstly reported for man in Ethiopia, too. Previous studies from human diarrhoeal outpatients in Addis Ababa show *S.* Typhi serogroup B, C, D and E whose source of infection may be contaminated food (Mache and Megistu, 1998). Concerning the slaughtered abattoir cattle *S.* Anatum and *S.* Dublin have been isolated. This could but must not have been linked to the consumption of raw meat by the abattoir personnel, which excreted the same serovars. These serovars were also isolated from the abattoir workers and children in Zimbabwe (Chambers, 1977). *S.* Meleagridis was also detected from the stool of abattoir workers. Its origin can only be justified by other contaminated food products, as it was not identified in any sample of the abattoir cattle. It should be pointed out that there was not any case of contamination of the 330 beef cut samples by *S.* Meleagridis.

Findings of *Salmonella* in minced beef from the supermarkets showed *S.* Anatum, *S.* Dublin and *S.* Saintpaul as the most frequent serotypes. The dominance of *S.* Anatum and *S.* Dublin in minced beef is in agreement with the work of Molla et al. (1999), concerning the same matrix. The source of the two serotypes found in minced beef from supermarkets could have been from the Addis Ababa abattoir because these strains were isolated from samples collected at the same slaughterhouse. On the other hand *S.* Saintpaul is a new serovar to be reported in Ethiopia. The origin of *S.* Saintpaul from Kaliti and Kara abattoirs supplying meat to supermarkets to be processed into minced beef was clearly shown in this study. From the point of view of tracing back it seems very important that no minced beef from Addis Ababa slaughterhouse contained *S.* Saintpaul because this serotype was not isolated at the slaughterhouse itself. However, *S.* Saintpaul has been well documented from two other abattoir studies (Adesiyun and Oni, 1980; Shrama and Joshi, 1992). There was no difference in overall frequency of *Salmonella* between the three abattoir sources except the difference in frequency of serotypes. In future there should be designed a study covering all meat products with a considerable *Salmonella* contamination risk to get more detailed information about the *Salmonella* infection chains in Ethiopia.

From the viewpoint of food hygiene and therapy of salmonellosis the sensitivity of *Salmonella* isolates to commonly used antimicrobial agents is of public health significance. In the present study 98 *Salmonella* strains (namely *S.* Anatum, *S.* Dublin, *S.* Meleagridis, *S.* Muenchen, *S.* Saintpaul and *S.* rough form) isolated from the abattoir cattle samples, slaughterhouse personnel and minced beef from supermarkets have been tested for resistance against 17 antimicrobials. All *Salmonella* strains showed sensitivity against the 17 drugs in the following concentration: Amikacin (AK 30 µg), Ampicillin (AMP 10 µg), Cefuraxolin (CXM 30 µg), Chloramphenicol (C 30 µg), Colistin (CT 10 µg), Enrofloxacin (ENR 5 µg), Furazolidon (FR 100 µg), Gentamycin (CN 10 µg), Kanamycin (K 30 µg), Nalidixin acid (Na 30 µg), Neomycin (N 10 µg), Polymyxin B (PB 300 IU), Streptomycin (S 25 µg), Sulphamethoxazol/Trimethoprim (SXT 25 µg), Sulphonamide (SU 300 µg), Tetracycline (TE 300 µg) and Trimethoprim (W 25 µg). This is a clear indication that most of the antmicrobial drugs are rarely utilized in cattle hence no drug resistance was observed antimicrobial testing. Preventing of development of resistance in *Salmonella* in food of animal origin does not only imply that antibiotics should never be administered to control subclinical *Salmonella* infection, but also that the use of antibiotics for food of animal origin in general should be to the least possible extent. Special care should be taken for the classes of antibiotics used for treatment of human *Salmonella* infections, notably flouroquinolones and aminoglycosides. Basing on the information presented above, it is important that the prudent use of fluoroquinolones in the Ethiopian veterinary field should continue with the idea that their application should maximize the therapeutic effect and minimize the emergence of resistance as documented by Malorny et al. (1999):

On the other hand recently research work demonstrated a rapid growing multidrug-resistance for many *Salmonella* strains isolated from livestock and humans in many regions of the world. The predominant serovars isolated of foods of animal origin, mainly *S.* Anatum and *S.* Dublin, have developed resistance patterns to streptomycin, neomycin and tetracycline (Adesiyun and Oni, 1980; Shrama and Joshi, 1992). From abattoir investigations carried out in Brazil the dominant strain from meat and meat products of cattle origin was *S.* Dublin too and this serotype was resistant to tetracycline, chloramphenicol, kanamycin, ampicillin, nalidixic acid, gentamicin and trimethoprim-sulmethoxazole (Composs et al., 1989). Other authors stated that the transition of

### Antimicrobial sensitivity of *Salmonella* isolates

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Regarding the given facts salmonellosis is of public health significance, because the probability of eating meat or meat products contaminated with *Salmonella* must be taken into account. Prevention measures must start at the level of animal production. Reduction of the prevalence of *Salmonella* should be based on good production practices (GPP) which includes biosecurity, cleaning, disinfection and animal hygiene. This GPP should furthermore be incorporated in food production, transport and selling as commodity food safety/quality assurance programmes. Integral for the success of interventions at the animal production level are in-depth understanding of the epidemiology and microbial characteristics of specific pathogens, animal identification and diagnostics. At the consumption end of the continuum, the public, especially food handlers and consumers, need education in safe handling of food. Furthermore, recommendations about important measures that reduce the general risk of infection from foods should be published. These consultation includes guidelines regarding the storage, handling, use and preparation of meat and meat products.

# 6. CONCLUSIONS AND RECOMMENDATIONS

1. Concerning the "cow-chain" the prevalence of *Salmonella* in single samples is relatively low in the faeces (2.1 %) and mesenteric lymphnodes (4.2 %) of the Ethiopian abattoir cattle in comparison to the *Salmonella* loads of beef cuts (9,8 % and 11.9 % respectively) and minced beef from supermarkets (7.9 %). Apart from these sources the level of the *Salmonella* prevalence in stool samples of slaughterhouse workers (6.0 %) lies between these two groups.
2. The highest prevalence of *Salmonella* in beef cuts was found in cattle being slaughtered in poor condition. To reduce the *Salmonella* burden to the public it is therefore essential to perform intensive live inspection and to slaughter only healthy animals.
3. The data from bacteriological analysis of beef cut samples from abdomen (9.8 %) and diaphragm (11.9 %) show that there must have been severe additional contaminations during the slaughter process, mainly during the step of skinning.
4. In 6 % of the human stool samples from the slaughterhouse workers *Salmonella* was isolated. Theoretically, the employees play an important role as a source for *Salmonella*

contamination. On the other hand *S.* Meleagridis was only found in human faeces samples and this serotype was never isolated from the "cow chain".

5. Although the level of the *Salmonella* load of minced beef from supermarkets (7.9%) does not exceed the *Salmonella* burden of the original beef cuts, there arises a severe risk for the consumer of raw beef.
6. The most frequent isolated *Salmonella* serotypes in cattle/beef samples were *S.* Dublin and *S.* Anatum. *S.* Muenchen and *S.* rough form were found less frequently. *S.* Meleagridis was only detected in human stool and *S.* Saintpaul in minced beef not originating from Addis Ababa slaughterhouse.
7. None of the 98 tested *Salmonella* strains showed resistance against 17 antimicrobial substances.

The following recommendations must be considered generally and are important to improve the hygienic status of Ethiopian beef:

- Separation of healthy and sick or suspected animals before slaughter and avoidance of stress to the animals, strict separation in the slaughterhouse between "unclean" and "clean" area.
- Environmental factors such as insects, rodents and avian vectors must be under control because they are involved in the infection of herds as well as in the contamination of processing areas and carcasses.
- During slaughter, Good Manufacturing Practices should be followed which include cleaning and disinfecting of knives and aprons, hand washing, avoidance of contact between carcasses and other surfaces and optimal cleaning education and motivation of slaughterhouse personnel and adequate control by competent official authority.

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# 8. APPENDICES

## Appendix 1.

**Positive cattle pooled samples of mesenteric lymphnodes and faeces**

**Data worksheet**

| Pooled ID | Me*S*.Ln | Faeces | Status | Trans | Cond | Antibiogram | Serovar |
|---|---|---|---|---|---|---|---|
| 1 | p | n | p | v | 2 | | |
| 2 | p | n | p | v | 1 | | |
| 3 | p | n | p | v | 2 | | |
| 4 | p | n | p | f | 1 | | |
| 5 | n | n | n | r | 2 | | |
| 6 | n | n | n | f | 2 | | |
| 7 | n | n | n | r | 2 | | |
| 8 | n | n | n | f | 1 | | |
| 9 | n | n | n | v | 2 | | |
| 10 | n | n | n | v | 2 | | |
| 11 | n | n | n | v | 1 | | |
| 12 | n | n | n | f | 1 | | |
| 13 | n | n | n | v | 1 | | |
| 14 | n | n | n | v | 0 | | |
| 15 | n | n | n | f | 1 | | |
| 16 | n | n | n | v | 1 | | |
| 17 | n | n | n | r | 1 | | |
| 18 | n | n | n | r | 2 | | |
| 19 | n | n | n | f | 2 | | |
| 20 | n | n | n | f | 2 | | |
| 21 | n | n | n | f | 2 | | |
| 22 | p | n | p | f | 2 | | |
| 23 | n | n | n | f | 2 | | |
| 24 | n | n | n | f | 1 | | |
| 25 | n | n | n | f | 2 | | |
| 26 | n | p | p | v | 3 | | |
| 27 | p | n | p | v | 3 | | |
| 28 | n | n | n | v | 3 | | |
| 29 | n | n | n | v | 2 | | |
| 30 | n | n | n | r | 2 | | |
| 31 | p | n | p | r | 2 | | |
| 32 | p | n | p | r | 2 | | |
| 33 | n | p | p | v | 1 | | |
| 34 | n | p | p | r | 1 | | |
| 35 | n | p | p | r | 3 | | |
| 36 | n | n | n | v | 1 | | |
| 37 | n | n | n | v | 1 | | |
| 38 | n | n | n | v | 2 | | |
| 39 | n | n | n | v | 2 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 40 | n | n | n | v | 2 | | |
| 41 | n | n | n | v | 2 | | |
| 42 | n | p | p | v | 2 | | |
| 43 | n | n | n | v | 2 | | |
| 44 | n | n | n | r | 1 | | |
| 45 | n | n | n | r | 1 | | |
| 46 | n | n | n | r | 1 | | |
| 47 | p | n | p | r | 1 | | |

## Appendix 2

**Created pool for the abdominal and diaphragmatic muscles**

**Data work sheet**

| Pooled ID | A.M | D.M | Status | Serotype | Antibiogram |
|---|---|---|---|---|---|
| 1 | n | n | n | | |
| 2 | n | n | n | | |
| 3 | p | n | p | | |
| 4 | n | n | n | | |
| 5 | p | p | p | | |
| 6 | n | n | n | | |
| 7 | n | n | n | | |
| 8 | p | p | p | | |
| 9 | n | n | n | | |
| 10 | n | p | p | | |
| 11 | n | n | n | | |
| 12 | n | n | n | | |
| 13 | n | n | n | | |
| 14 | n | n | n | | |
| 15 | n | n | n | | |
| 16 | n | p | p | | |
| 17 | n | n | n | | |
| 18 | n | p | p | | |
| 19 | n | n | n | | |
| 20 | p | p | p | | |
| 21 | p | p | p | | |
| 22 | p | p | p | | |
| 23 | p | n | p | | |
| 24 | p | n | p | | |
| 25 | n | n | n | | |
| 26 | n | n | n | | |
| 27 | n | n | n | | |
| 28 | n | n | n | | |
| 29 | n | p | p | | |
| 30 | n | p | p | | |
| 31 | p | p | p | | |

| | | | | | |
|---|---|---|---|---|---|
| 32 | p | p | p | | |
| 33 | p | n | p | | |
| 34 | n | p | p | | |
| 35 | p | p | p | | |
| 36 | p | p | p | | |
| 37 | n | n | n | | |
| 38 | n | p | p | | |
| 39 | p | n | p | | |
| 40 | p | n | p | | |
| 41 | p | p | p | | |
| 42 | p | p | p | | |
| 43 | n | p | p | | |
| 44 | n | p | p | | |
| 45 | p | p | p | | |
| 46 | p | n | p | | |
| 47 | n | p | p | | |

## Appendix 3

**Abattoir Personnel Positive Results**

| SA | | Status | Antibiogram | Serotype | | | |
|---|---|---|---|---|---|---|---|
| 10 | A010 | p | NR | | SD | | |
| 32 | A032 | p | NR | SA | | | |
| 41 | A041 | p | NR | | | SME | |
| 52 | A052 | p | NR | | | SME | |
| 70 | A070 | p | NR | | SD | | |
| 80 | A080 | p | NR | | | SME | |
| 98 | A098 | p | NR | | SD | | |
| 102 | A102 | p | '' | SA | | | |
| 129 | A129 | p | '' | | SD | | |
| 148 | A148 | p | '' | SA | | | |
| 159 | A159 | p | '' | | | SME | |
| 168 | A168 | p | '' | SA | | | |
| 176 | A176 | p | '' | | | SME | |
| 178 | A178 | p | '' | | | | SR |
| 224 | A224 | p | '' | SA | | | |
| 239 | A239 | p | '' | | | | SR |
| 272 | A272 | p | '' | SA | | | |
| 274 | A274 | p | '' | SA | | | |
| | | | | **7SA** | **4SD** | **5SME** | **2SR** |

N = Negative ; P = Positive
SA = *Salmonella* Anatum
SD = *Salmonella* Dublin
SME = *Salmonella* Meleagridis
SMU= *Salmonella* Muenchen
SRF = *Salmonella*.rough form
NR = No Resistance

Appendix 4

**Salmonella isolation in minced beef from supermarkets**

| Supermarket ID No | Status | Source | Storage | Antiboigram | No. *of Salmonella* isolates | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | SA | SD | Ssp | SR |
| 1 | p | 2 | 1 | NR | | 1 | | |
| 2 | n | 1 | 2 | NR | | | | |
| 3 | p | 3 | 1 | NR | | | 1 | |
| 4 | p | 1 | 1 | NR | 1 | | | |
| 5 | p | 2 | 0 | NR | | | 1 | |
| 6 | p | 2 | 1 | NR | | | 1 | |
| 7 | p | 1 | 1 | NR | 1 | | | |
| 8 | p | 2 | 2 | NR | | | 2 | |
| 9 | p | 1 | 1 | NR | 3 | 1 | | 1 |
| 10 | p | 2 | 1 | " | 2 | | 1 | |
| 11 | n | 2 | 0 | " | | | | |
| 12 | n | 2 | 1 | " | | | | |
| 13 | n | 2 | 1 | " | | | | |
| 14 | p | 1 | 1 | " | | 1 | | |
| 15 | p | 1 | 0 | " | 1 | | | |
| 16 | n | 1 | 2 | " | | | | |
| 17 | p | 1 | 1 | " | 1 | | | |
| 18 | p | 3 | 1 | " | | | 2 | |
| 19 | p | 1 | 0 | " | 2 | | | |
| 20 | p | 2 | 1 | " | | | 1 | |
| 21 | p | 1 | 1 | " | | | | |
| 22 | n | 2 | 1 | " | 1 | 1 | | |
| **Total** | | | | | **12** | **4** | **9** | **1** |

SA = *Salmonella* Anatum	1= Addis Ababa abattoir

SD = *Salmonella* Dublin	2 = Kaliti abattoir

SSp = *Salmonella* Saintpaul	3 = Kara abattoir

SRF = *Salmonella* rough form

NR= No Resistance

Appendix 5

## *Questionnaire Specimen*

### A. Abattoir

- **Pre-Slaughter conditions**

1. Origin of animals
2. The Mode of Transport
   a. Vehicle b. Rail c. By foot
3. Disease History from source of animals
4. Age groups of animals brought for slaughte
   a. Young b. Juveniles c. Adults
5. Identification of animals
   a. Tatoo b. Ear tags c. None
6. The number of animals slaughtered in a day
7. Against which diseases are animals screened before slaughter
8. Who carries out the ante-mortem inspection?
   a. Veterinarians b. Animal Health Technicians c. None

- **Slaughter Facilities**

9. The design and facilities in the abattoir
10. The general surrounding in the abattoir
11. The facilities used during slaughter of animals
12. The type of protective clothing used by the personnel in the abattoir
13. What is the source and quality of water used?
    a. Tap water b. Hand carried c. Borehole d. wells
14. How are the animals slaughtered?
    a. Mechanized b. Manual
15. Who carries out the post-mortem inspection?
    a. Veterinarians b. Animal Health Technicians c. None
16. How are the condemned specimens disposed?

17. The disposal of waste

    a. Incenerator b. Pit-holes c. Open disposal

18. What are the commonest conditions at meat inspection?

- **Post-Slaughter handling and disease status**

19. The type of laboratory analysis in case of a suspected diseases
20. Any reported disease cases from the personnel
21. The type of storage facilities

    a. Cold rooms b. Fridges c. none

22. How is the meat transported to the supermarkets and butcher shops?
23. To which supermarkets and butcher shops is the meat supplied in the city?

**B. Supermarket and butcher shops**

1. The source of meat
2. What is the mode of transportation of meat and meat products

    a. Open Vehicles b. Closed Vehicles c. Closed and refrigerated d. By hand

3. The type of storage facilities

    a. Cold room b. Refrigerators c. None

4. What are the types of meat processing carried out?
5. The type of cutting facilities
6. How is the packaging done
7. Any previous complaints from clients on illness contacted on consumption of meat and meat products
8. Is there any disease screening for the personnel? Which ones?
9. The type of protective clothing used by the personnel
10. Is there any inspection by the Public health personnel
11. Are there any specimens sampled for laboratory analysis?

## 10. Signed Declaration Sheet

I, under signed, declare that the thesis is my original work and has not been presented for a degree in any University.

Name: Charles Nyeleti

Signature: ______

Date of Submission: 15TH November, 1999

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

AAU: DR. BAYLEYEGN MOLLA ______

FUB: PROF. DR. GOETZ HILDEBRANDT ______

FUB: DR. JOSEF KLEER ______