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A STUDY ON PREVALENCE AND EPIDEMIOLOGICAL KEY FACTORS OF SALMONELLA SPECIES IN SELECTED POULTRY FARMS IN DEBRE ZEIT, ETHIOPIA

by

Marosi A. Molomo

January, 1998

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A thesis submitted in partial fulfilment for the degree of
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THE PREVALENCE AND EPIDEMIOLOGICAL KEY FACTORS OF SALMONELLA SPECIES IN SOME SELECTED LARGE COMMERCIAL AND SMALL HOLDER POULTRY FARMS IN DEBRE ZEIT, ETHIOPIA

by

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Dedication

This work is dedicated to my beloved husband Koenane and my daughters Senate and Liako Moshoeshoe for their love and support.

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LIST OF ABBREVIATIONS

AVID	= Arbeitskreis für veterinärmedizinische Infektionsdiagnostik
BgVV	= Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin
BPLS- agar	= Brilliantgreen- Phenolred- lactose- saccharose agar
BPW	= Buffered peptone water
ELISA	= Enzyme- Linked Immunosorbent Assay
H_2S	= Hydrogen sulphide
LPS	= Lipopolisaccharide
MAC	= MacConkey agar
RV- broth	= Rappaport - Vassiliadis broth
SAT	= Serum Agglutination Test
Spp	= Species
Subsp.	= Subspecies
TBG- broth	= Tetrathionate- brilliant - green- bile broth
TSI- agar	= Triple sugar iron agar
WBT	= White Blood Test
XLD	= Xylose- lysine- desoxychocolate agar
XLT_4	= Xylose lysine tergitol 4 agar

LIST OF APPENDICES

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ABSTRACT

A cross-sectional and a longitudinal study were undertaken for a period of 5 months to identify current salmonellae serotypes prevailing in selected poultry farms in Debre Zeit, Ethiopia. Serotypes of particular interest were S. Enteritidis and S. Typhimurium which are of public health importance and S. Gallinarum which is of economic significance. In the cross-sectional study, 8 farms with 20 flocks of chickens were investigated. The farms were subdivided according to ownership, the large state farms, large private farms and small private farms. These farms were labeled from A to H and flocks in farms were labeled accordingly. Historical data of the last 5 years were obtained and analyzed for farm A.

A questionnaire survey was conducted in all farms. The questionnaire was subdivided into three parts: biosecurity measures, health and hygiene status and management. All answers (variables) applicable to all farms were subjected to a scoring system per individual farm and to a scoring system of the three subgroup farms. Seventeen flocks were tested for the presence of S. Gallinarum (fowl typhoid) using commercial stained antigen. Sample size were 30 chickens from each flock. The remaining three flocks were too young for the test. A total of 244 environmental and tissue samples were also collected from all the flocks and processed using cultural techniques.

For the longitudinal study, farm E was selected. The farm contained positive reactors for fowl typhoid and also S. Enteritidis was isolated from the nest boxes in the cross-sectional part. Eggs from this farm were followed to the hatchery to establish whether salmonellae were transmitted from eggs to chicks via the hatchery from this flock. Two farms that received one day old chicks from this hatchery (farm E1.1 and E1.2) were followed up until the end of rearing period. A total of 139 environmental and tissue samples were collected and processed, including samples from the hatchery.

For culturing buffered peptone water (BPW) was used for pre-enrichment, Rappaport-Vassiliadis (RV) and Tetrathionate-brilliantgreen-bile (TGB)- broths were used for selective enrichment. Plating out was done on Brilliantgreen-Phenolred-lacrose-saccharose (BPLS) and Xylose-lysine-desoxychocolate (XLD) agars. Biochemical characterization of suspicious colonies was done using Triple sugar iron (TSI) agar, lysine decarboxylase broth and Simmon citrate agar. The suspected salmonella colonies were further confirmed serologically by group-specific salmonella anti- sera. Isolates showing positive reaction to any of these anti-sera were further identified by testing sera specific for groups B and D. Finally positive salmonella isolates were stored in nutrient agar and sent to the BgVV-Berlin (Salmonella reference laboratory) for final confirmation. Antimicrobial test using Amikasin (AK 30), Ampicillin (AMP 10), Cefuraxolin (CXM 30), Chloramphenicol (C 30), Colistin (CT 10), Enrofloxacin (ENR 5), Furazolidon (FR 100), Gentamycin (CNN 10), Kanamycin (K 30), Nalidixin acid (Na 30), Neomycin (N 10), Polymyxin (PB 300), Streptomycin (S 25), Sulphamethoxazol/Trimethoprim (SXT 25) Trimethoprim (W 25) was performed at the BgVV.

Historical data analysis showed significant differences in mortality ($P < 0.05$) caused by salmonella infection between months of the 5 year study period. However, regression square (R^2) showed a 47% of variation observable mortality over the years. Economic

losses due to salmonellosis in infected poultry in regards to high mortality and to a considerable decrease in egg production from 1992-97 could be demonstrated.

Farm scores were calculated by dividing the range by the number of class intervals. Farms C, D, E, F and G were graded very good or good respectively. Farms A, B and H were graded very bad or bad. These farms are large private and state owned farms. Fowl typhoid test identified only three flocks positive to S. Gallinarum (flocks B/1, D/1 and E/1) at time of the investigation. These farms were also positive to S. Enteritidis, and S. Anatum on culture techniques. Twenty suspicious salmonella were sent to BgVV for confirmation. Ten were confirmed as salmonella positive species. S. Typhimurium was not isolated. From the ten confirmed salmonella positive isolates, 7 were from environmental sources including dust inside pens, litters and nest boxes. From these 7 strains, S. Enteritidis was prevalent in four samples i.e. 1 sample from dust inside pen, 2 litter samples and in 1 nest boxes sample. S. Anatum was isolated in another dust inside pen sample and S. Uganda from another source of dust inside pen. From 28 tissue samples investigated, including liver and caecal samples three samples were salmonella positive. S. Enteritidis was isolated in liver and in caecal sample, the third isolate was a rough strain from the liver samples.

From the longitudinal study, 4 isolates originating from both farms were sent for confirmation and all three of these from farm E1.1 were confirmed salmonella positive. All isolates were from environmental samples including drag swab, surroundings and litter. S. Enteritidis was isolated from the drag swab and swab samples of the surroundings while S. Uganda was identified in the litter sample.

For both studies, antimicrobial tests were done on 8 strains of S. Enteritidis, 1 of S. Anatum, 2 of S. Uganda , and 2 rough strains. 30% of the isolates were sensitive to all drugs (2 S. Uganda, 1 S. Enteritidis and 1 rough strains). 6 strains of S. Enteritidis (75%) were resistant to tetracycline while 1 strain (12.5%) was resistant to Nalidixin acid. The S. Anatum strain showed resistance to tetracycline, sulphamethoxazol/trimethoprim and trimethoprim. The results of this study showed that *Salmonella* could be isolated from all production levels, i.e. layers, broilers and even in breeder flocks. The most prevalent serovar detected was S. Enteritidis. These strains are more resistant to tetracycline. A potential public health hazard due to S. Enteritidis is obvious. The public health importance is also indicated by the identifications of S. Uganda and S. Anatum. No S. Typhimurium was detected. S. Gallinarum were prevalent in 18% of the investigated flocks. The potential of economic loss due to salmonellosis is indicated. Further studies on the prevalence of *Salmonella* species in increasing number of flocks and focusing mainly on the major sources of infection are required in this area.

1. INTRODUCTION AND OBJECTIVES

Ethiopia is found in the eastern part of Africa and is bounded in the northeast by Eritrea, Djibouti in the east, southeast by Somalia, in the southwest by Kenya and in the west and northwest by Sudan. The area of the country is 1,128,176sq km (435,606sq.) (Microsoft Encarta, 1994).

The economy of the country heavily depends on the earnings from the agricultural sector. Traditional agriculture by primitive methods, including the raising of livestock comprising of cattle, horses and donkeys, camels, chickens etc, is the most characteristic form of Ethiopian economic activity (Microsoft Encarta, 1994). Poultry production in Ethiopia is characterized by small scavenging flocks of local birds kept by individual households. Statistical data of 1990 suggest that there are 58 million birds producing 79,120 tons eggs and 76,560 tons meat. Per capita consumption is estimated to be 40 eggs and 1.75 kg chicken meat per annum (FAO/World Bank, 1993). Like in most developing countries, poultry production in Ethiopia plays a very important role as being one of the main sources of protein and income generation (Alamargot, 1987).

Poultry production is concentrated in the temperate zones of the country; the administrative regions of Shewa, Sidamo, Gojam and Wello. Regions like Illubalor, Gamo Gofa and Bale have limited production potentials. Temperatures outside these altitudes hinder poultry production. Commercial flocks run by agricultural universities and colleges, children's villages, urban dwellers and peasant associations as well as the state, are concentrated around Addis Ababa and Debre Zeit (FAO/ World Bank, 1993).

An unpublished report of the Faculty of Veterinary Medicine, Addis Ababa University (FVM Debre Zeit, Ethiopia) indicates that there are three types of poultry production systems, namely Large and Small scale commercial and Traditional (back yard) The large scale poultry production system is owned by the state. There are three major poultry farms owned by the state in Addis Ababa and Debre Zeit; namely Shola, Lemlem and Dembi farms. Shola and Lemlem farms have a holding capacity of 60,000 layers, and 400,000 broilers per annum. Dembi farm is the largest of the three and is composed of a parent flock, hatchery and rearing quarters with a capacity of 1.7 million day old chicks, 150,000 layers and 234,000 pullets. The majority of layer chicks are imported as day olds. Feeders and drinkers are automated.

The small scale system mainly deals with broilers with a capacity of a 100 to 1000 per farm. The feed supply is local for all systems. The traditional chickens live on scavenged feed. An average household has 6-10 birds producing 55 and 80 eggs with an average egg weight of 45g. Mature body weight ranges from 1.0 kg for female chickens to 1.5 kg for male. The equipment like drinkers, feeders are usually hand made and traditional in most cases.

The major poultry diseases/conditions of these farms are Newcastle Disease, fowl cholera, internal and external parasites, nutritional deficiency, coccidiosis, mycoplasmosis and salmonellosis (Alamargot, 1987). The importance of *Salmonellae* in poultry lies in the disease they cause in poultry and the public health risks posed by certain members of these bacteria (Coetzee *et al.*, 1993). Poultry constitutes an important reservoir of *Salmonellae*. This organism is often observed in intensive poultry farms (Buxton and Fraser, 1977).

Salmonellosis caused by S. Gallinarum is considered to be an economically important disease in poultry worldwide (Bean and Griffin, 1990; Bryan, 1988; Humphrey and Mead, 1988). Frequently isolated serovars from human cases are S. Typhimurium and S. Enteritidis (Hassan *et al.*, 1990). Usually infection of animals with *Salmonella* may not be clinically apparent. In its subclinical form, the animal harbors *Salmonella* in the intestines and in lymphnode tissues intermittently and may excrete the agent in its faeces hence serving as the main reservoir of infection. Clinical salmonellosis, on the other hand, can result in serious disease that manifests itself as septicaemia, acute or a chronic enteritis. *Salmonellae* are often the etiological agents of food-borne salmonellosis. Over the years, the incidence of food poisoning in the human population due to *Salmonella* has increased dramatically in Europe and in other parts of the world, and poultry are recognized as a major source of infection in many cases (Silliker, 1980; Mead, 1990; Herenda *et al.*, 1994).

Isolation and identification of the causal organisms play a very important role in the diagnosis of *Salmonella* infection (Buxton and Fraser, 1977; WHO, 1994). Several techniques have successfully been applied, these consist of at least five stages (International Standard Organization (ISO), 1993): Pre-enrichment or resuscitation in liquid media, selective enrichment in liquid media, plating on selective agar media, screening of suspected colonies on media revealing key biochemical characteristics of the organism and serological tests with the use of commercially available polyvalent, group and mono-specific *Salmonella* anti-sera.

Serological methods have been developed to detect flocks infected with S. Gallinarum. These are the Serum Agglutination Test (SAT) and Whole Blood Test (WBT) with a stained antigen (WHO, 1994). Furthermore, a number of different rapid methods such as Enzyme-Linked Immunosorbent Assays (ELISA) techniques are currently used world-wide to detect *Salmonella* infection. (WHO, 1994).

For epidemiologists, whose main task is finding the source of infection, it is important to know serotypes causing the epidemics (Ketchum, 1988). Bouzoubaa *et al.* (1991) in their investigation in Morocco found that village chickens were positive to S. Pullorum and S. Gallinarum. This implicates that village chickens can act as reservoirs. In Ethiopia, as mentioned earlier, the majority (99%) of chickens are local birds and the intensive system of poultry rearing is currently expanding. The chances of spreading *Salmonella* organisms between the traditional and modern systems are therefore high.

In Ethiopia, Teshome and Mebratu (1988) detected S. Pullorum infection from 40% of broiler breeder stock by SAT and about 80% of samples taken from dead chickens from those breeder stocks at 4-10 days were culturally positive for S. Pullorum.

Salmonellosis is one of the most important diseases that hinder the large commercial farms to handle parent stock in Ethiopia. These breeder flocks are imported as day old chicks and they do not complete their production cycle due to *Salmonella* infection. Despite frequent outbreaks of salmonellosis in commercial poultry farms since 1985 (Mohammed, personal communication), a thorough epidemiological investigation of the disease focusing on sources of infection has not yet been conducted. In addition, serotypes that are prevailing in these poultry farms have not been determined recently. It is, therefore of great importance to know which serotypes are present in the flocks that in turn help to identify the routes of transmission and to envisage cost-effective control measures. The study will serve as a starting tool to conduct further investigations on salmonellosis in the study area; not only for the economic benefits of the poultry production industry but also in addressing its public health concern.

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

1 - Conduct a questionnaire survey and assess the prevalence of *Salmonella* infection in selected poultry farms in Debre Zeit, Ethiopia.

2 - Isolate and serotype *Salmonellae* in selected farms in Debre Zeit, and ultimately determine the current most prevailing *Salmonella* serovars in the study population.

3 - Estimate possible risk factors in the transmission of *Salmonella* as vital create tools to formulate effective control and preventive strategies, the clue to S. Enteritidis and S. Typhimurium (causes of major public health concern) and S. Gallinarum-Pullorum (due to its economic significance).

2. LITERATURE REVIEW

2.1. The Genus *Salmonella*

2.1.1. Taxonomy

Salmonellae are members of the *Enterobacteriaceae* family (Ketchum, 1988; Biberstein and Zee, 1990; Zeidler, 1996). The genus is divided into two species; *S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies; *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica*. *S. bongori* is regarded as single species (Le Minor and Popoff, 1987; Reeves *et al.*, 1989). Serovars belonging to *S. enterica* subsp. *enterica* are designated by a name usually related to the geographical place where they were first isolated (Popoff *et al.*, 1996). Such names are written in roman letters (not italicized), and the first letter in capital; whereas serovars belonging to other subspecies are designated by their antigenic formula, following the subspecies name.

2.1.2. Morphology

Salmonellae are short rods of 2-4μm in length and 0.5μm in width. However, culture was showed to change them into pleomorphic forms. *Salmonellae* possess peritrichous flagella with the exception of S. Gallinarum which are non motile organisms. Some strains form capsules. They are Gram-negative organisms (Buxton and Fraser, 1977; Old, 1989; Seifert, 1996).

2.1.3. Antigenic structure

Salmonella has three antigens namely "O", "H", and "Vi". The "O", or somatic antigens are part of the Lipopolisaccharide (LPS) component of the cell wall that also contain lipid A and a core portion. They consist of repetitive oligosaccharide units of which the type order, and repetition of sugar differ between serovar. There are 67 different "O" antigens identified by Arabic numerals, of which some occur singly while others in combination (Le Minor, 1984). There are smooth and rough strains of the "O" antigens. The smooth strains possess a complete oligosaccharide "O" specific side chains and virulence properties. The rough strains

are results of incomplete oligosaccharide "O" specific side chains (Le Minor, 1984; Old, 1989). The other antigens assayed in the Kauffmann - White - scheme are the flagellin protein (H- antigens). The flagella enable *Salmonella* to be motile. These flagella are composed of protein (flagellin). The "H" antigens have two phases; phase 1 which is identified by letters of the alphabet and phase 2 is identified by numbers respectively. "Vi" antigens are associated with the virulence of S. Typhi for mice and occur on a few other serotypes (Old, 1989).

In order to establish a complete antigenic composition of any *Salmonella* serovar, antigens of both flagella phases as well as the "O" must be known (Le Minor, 1984). Classification by Kauffmann-White diagnostic scheme by means of agglutination test has been indicated on Table 1.

Table 1: Classification of *Salmonellae* showing representative serotypes (Kauffmann-White scheme) (Kelternborn, 1992)

			Flagella antigens ("H")	
Group	Serovar	Somatic	phase1	phase2
B	S. Typhimurium	1,4,5,12	i	1,2
D1	S. Enteritidis	1,9,12	g,m,[f] [p]	[1,7]
D1	S. Gallinarum / Pullorum	1,9,12	-	-
E1	S. Uganda	3,10, [15]	$1,z_{13}$	1,5
E1	S. Anatum	3,10, [15] [15, 34]	e,h	1,6

- = can only be detected in some special conditions
[] = can be present or not.

2.1.4. Diagnostic methods used to detect *Salmonella*

2.1.4.1. Isolation of *Salmonella* and sampling procedures

Isolation begins with the collection of samples. Great care is required when collecting samples. With live birds, all samples of the environment and feed must be collected aseptically in order to prevent cross contamination. In poultry, sampling strategy plays a very important aspect in the isolation of the bacteria. Lahellec and Colin (1985), in their longitudinal study, collected samples from the first day in the hatchery and poultry houses, then weekly in the houses until processing. They detected very marked increase in the incidence of *Salmonella* positive samples occurring between hatching and first week of the rearing period, and between the end of rearing and processing. According to these works, a negative result does not mean a farm is *Salmonella* free, because some serotypes (serovars) may persist in the environment without being isolated by the available monitoring procedures. Earlier studies showed that a higher percentage of dust and eggbelt samples were positive for *Salmonella* than faecal samples (Poppe *et al.*, 1991)

There are different laboratory cultivation procedures. ISO (1993) recommended three stages of cultural procedures, i.e. pre-enrichment, enrichment and selective plating. The type of media in use depends on the laboratory that conduct the procedure. Waltman *et al.* (1993) described that most laboratories use buffered peptone water (BPW) for pre-enrichment, Rappaport Vassiliadis (RV), selenite and tetrathionate (TGB) broths for enrichment and for selective media mostly Brilliantgreen-Phenolred-lactose-saccharose (BPLS), Xylose-lysine-

desoxichocolate (XLD), Xylose lysine tergitol 4 (XLT4), MacConkey (MAC) agars. These media are also recommended by ISO, (1993) and WHO (1994). Poppe *et al.* (1996) combined RV and Rambach agar for the isolation of *Salmonella*.

The pre-enrichment step in non-selective media is to regain those *Salmonellae* that are stressed due to changes in their environment. They need to be resuscitated to repair any damaged *Salmonella* cells. Furthermore pre-enrichment is useful for dilution of toxic or inhibitory substances (Bailey and Cox, 1996). However, direct plating of samples like liver, spleen, heart, yolk sac, ovary on non-selective agar is recommended for birds with systemic salmonellosis, whereas isolation from intestines require selective media (Mallinson and Snoeyenbos, 1989).

2.1.4.2. Cultural characteristics of the isolates

In aerobic and anaerobic conditions, *Salmonella* grow readily on ordinary media producing colonies indistinguishable from those of other *Enterobacteriaceae*. Most *Salmonellae* are prototrophic and grow in minimal-salts medium with a suitable carbon source (Old, 1989). Auxotrophic serotypes adapted to a single host species, and auxotrophic mutant strains in other serotypes, grow in a minimal medium supplemented with an appropriate growth factor (Old, 1989). Because *Salmonellae* are somewhat more dye-resistant than most *Enterobacteriaceae*, certain dyes such as malachite green and brilliant green may be incorporated in some media to render them selective for growth (Andrian and Delaat, 1979). On agar based media the colonies are fairly large with an average diameter of 2-3 μm, but, vary considerably in size. They may be circular and a less regular surface and a more effuse serrated edge or they may assume the vine-leaf form (Andrian and Delaat, 1979).

2.1.5. Identification of *Salmonella*

2.1.5.1. Biochemical characteristics

Salmonellae are characterized by fermentation of glucose, maltose, mannitol and sorbitol with the production of acid and gas; absence of fermentation of sucrose, salicin and adonitol and failure to produce indole, to hydrolyze urea or to deaminate phenylalanine and a positive methyl-red reaction and a negative Voges-Proskauer reaction (Andrian and Delaat, 1979). They produce hydrogen sulphide (H_2S), in iron contained media and are lactose non-fermenting (Biberstein and Zee 1990).

ISO (1993) indicated that several biochemical tests may be used in the identification of *Salmonella*. On triple sugar iron agar (TSI), 100% of *Salmonella* spp. ferment glucose, 91.9% produce gas, 91.6% produce H_2S. On lysine decarboxylase, 94.6% react positive whereas 98.9% show negative results on indole reaction, and 99% are negative to urea splitting, while 99.2% are negative to sucrose. Rusul *et al.* (1996) used TSI and Lysine decarboxylase broth for biochemical test. Other biochemical tests which are sometimes used to differentiate S. Pullorum to S. Gallinarum include fermentation of sugars such as dextrose, lactose, sucrose, mannitol, maltose etc (William *et al.*, 1980).

2.1.5.2. Serological identification

Characterization of *Salmonella* by "O"-, "H"-, and "Vi"-antigenicity, which used to be essential for serovar definition, has become the most important typing method, as more than

2000 serotypes are currently recognized (WHO, 1992; Popoff *et al.*, 1996). Serological typing using agglutination reactions is an effective tool for identifying the over 2000 serotypes of *Salmonella*. The Kauffmann-White scheme systematically assigns numbers and letters to the different "O", "H" and "Vi" (virulence) antigens to create distinctive serotypes (Ewing, 1986; Ketchum, 1988; Le Minor, 1988; Quinn *et al.*, 1995).

Based on antigenic analysis, Kauffmann's classification is still the most acceptable. It is based on slide agglutination tests with absorbed sera so that the content of "O" antigens and of phase 1 and phase 2 "H" antigens can be determined (Buxton and Fraser, 1977; Seifert, 1996). *Salmonella* identification is performed in order to determine the group to which they belong. The culture of *Salmonella* to be serotyped can be taken from TSI slants or nutrient agar (Buxton and Fraser, 1977).

2.1.5.3. Phage typing method

Phage typing is the characterization of bacteria based on susceptibility to infection by a defined panel of bacteriophages. It is normally the second method to be applied in the study of *Salmonella* epidemiology. Phage typing is limited to a few serovars, notably S. Typhi, S. Typhimurium, S. Dublin, S. Enteritidis, S. Heidelberg. In S. Enteritidis, as in S. Typhimurium, phage typing is the principal method of typing (Olsen *et al.*, 1993). Recently, *incN* plasmids have been shown to cause phage type conversion in a broader range of important phage types of S. Enteritidis (Threlfall and Cahart, 1993).

2.1.5.4. Biotyping

Biotyping provides a means of separating strains of a species based on their reaction differences in selected biochemical tests. It has been widely used for typing of bacteria but is not so common any more (Olsen *et al.*, 1993). The reason for this is partly that the stability of biotypes is considered to be insufficiently proven and that the method is very laborious (Le Minor, 1988). This method is not very discriminatory.

2.1.5.4. Antibiogram typing method

This typing method is rarely used alone as the typeability of strains of *Salmonella* from livestock is often low due to the relatively low prevalence of antibiotic resistant strains (Olsen *et al.*, 1993).

2.1.5.6. Molecular typing methods

These methods are available and in regular use both in public health and veterinary laboratories. These methods have been developed to supplement serotyping and phage typing which are in extensive use (Barrow, 1997). The predominance of certain phage types of S. Enteritidis in various countries makes further epidemiological sub- grouping necessary. Today, this is achieved by using **molecular typing methods**. For various bacterial species, plasmid profiling, the pattern of outer membrane proteins and LPS, the fingerprinting of total genomic DNA including ribotyping, and multilocus enzymes electrophoretic typing have proven very useful (Helmuth and Schroeter, 1994). When such methods have been applied to S. Enteritidis, they revealed a homogeneous, clonal structure in contemporary PT4 isolates. Furthermore they indicate that the clone observed today emerged from a heterogeneous population before the onset of epidemic (Helmuth and Schroeter, 1994).

2.1.6. Diagnostic methods used for the detection of *Salmonella* antibodies

Other serological methods for the detection of *Salmonella* infection in poultry have been developed. The Whole Blood Test (WBT) and Serum Agglutination Test (SAT) with a stained antigen have been used for identification of flocks infected with S. Gallinarum/ Pullorum (WHO, 1994). Fowl-typhoid agglutination test has been proven to give false positive results. Bergguist *et al.* (1973) suggested that bacterial culture is performed to confirm the presence of the serotype Gallinarum.

According to WHO (1994), ELISA test has been developed for the diagnosis of S. Enteritidis infections. Birds which are serologically positive may have ceased to excrete *Salmonella* although circulating immunoglobulin concentrations may remain high. At the same time, serologically negative birds may result from a recent infection causing excretion before immunoglobulin production is maximum or infection with less invasive serotypes than S. Enteritidis or any other. With an appropriate monitoring program recently infected birds stand a chance of being detected serologically. Newly hatched chicks are immunologically immature and do not respond serologically to the somatic lipopolisaccharide (LPS) antigens until 2 to 3 weeks of age. Following *Salmonella* infection the immunoglobulin concentration is elevated for 2 to 3 months. An immunoglobulin-M (IgM) response indicates recent infection. These methods are used to identify infected flocks rather than individual birds.

2.2. Epidemiology

2.2.1. Transmission routes

Salmonella species are ubiquitous geographically and zoologically and occur universally in wide range of species. It is most prevalent in areas of intensive animal husbandry especially of poultry and pigs (Sewell and Brocklesby, 1990). Some serotypes are relatively host specific while others affect a wide host range. Feral birds and rodents play important roles in interspecies dissemination of infection (Biberstein and Zee, 1990; Borland, 1995; Swamy *et al* 1996). *Salmonellae* are horizontally and vertically transmitted and usually cause a permanent flock infection (Mallinson and Snoeyenbos, 1989). The main route of infection before hatch is thought to be vertical transmission of the *Salmonella* from parent to progeny.

Poultry are known to be major reservoirs of *Salmonella* organisms (Hobbs and Gilbert, 1978). Researches have shown that *Salmonella* can be passed through the developing ovum to the egg, however, the exact mode of infection is not fully understood (Reiber and Conner, 1995). In hatchery, more *Salmonella* was found to spread in the hatcher and contaminate chicks after pip than through the egg shell or in the incubator (Cason *et al.*, 1993). Teshome and Mebratu (1988) isolated S. Pullorum from chicks during the rearing period. The chicks were born from 80% S. Pullorum positive broiler parent stock by SAT. In this case the mode of dissemination was most likely through egg transmission from infected parents. However, transovarian transmission occurs rarely (Bichler *et al.*, 1996; McLlroy, 1996). Rusul *et al.* (1996) isolated *Salmonella* from the processing plant.

It can happen that birds which are not infected during rearing period, can be found infected during transportation and processing and most likely the same serotypes can be found in the poultry houses and processing plants (Lahellec and Colin, 1985). The reservoir of infection is the intestinal tract of warm-blooded and cold-blooded animals. The majority of infected

animals become subclinical excretors. However, *Salmonella* can survive nine months or more in the environment in sites such as moist soil, water, faecal particles and animal feeds, especially in blood, bone and fish meal (Hirsh, 1990; Quinn *et al.*, 1995).

Infection occurs following the ingestion of viable *Salmonellae*. Disease may follow infection immediately in an animal already infected, or due to a change in the intestinal environment. As with S. Typhimurium, S. Enteritidis transmission can be present in the ovary and oviduct in a small percentage of clinically healthy birds. Systemic infection of birds with S. Enteritidis and S. Typhimurium increases the risk of intestinal contamination of eggs with the organism. In addition, contamination of egg shell can take place when the egg comes in contact with faecal and other materials contaminated with *Salmonella* (Shivaprasad *et al.*, 1990).

2.2.2. Host related risk factors

The outcome of the interaction between host and *Salmonella* depends upon the state of the colonization, resistance of the host, infectious dose and the particular species of *Salmonella* involved (Hirsh, 1990). Newly hatched chicks are more susceptible to *Salmonella* infection. Exposure of such young chicks to *Salmonella* organisms results in sporadic outbreaks of salmonellosis with mortality; however, most infections result in colonization without disease and can be followed by long-term carriages and shedding. The probability of colonization by *Salmonella* is greatly reduced by prior establishment of a normal flora and increased age of birds (Nurmi and Nuotio, 1994).

Adult chickens are more resistant to infection with *Salmonella*, and carrier rates in healthy birds are normally very low (Schiemann and Montgomery, 1992; Hassan *et al.*, 1990). Antibodies may develop in birds which have ceased to excrete *Salmonella* although immunoglobulin concentration may remain high. Also negative tests may result from a recent infection causing excretion before immunoglobulin production reaches maximum level to be detected (Hassan *et al.*, 1990; WHO, 1994). In a host, protection depends upon specific and non-specific immunological factors and microbiological defense mechanisms (Biberstein and Zee, 1990).

Live birds have been reported to carry a variety of *Salmonella* serovars varying up to 50 within a flock at any given time, with contamination levels ranging from 13.7 to 88.8% (Mackenzie and Bins, 1976; Higgins *et al.*, 1981; Bahatia *et al.*, 1986; Lahellec *et al.*, 1986; Jones *et al.*, 1991; Poppe *et al.*, 1991). Chauhan, (1993) indicated that poultry, turkey, quails, ducks, sparrows, pigeons can suffer from Pullorum disease. Fowl typhoid may affect birds of any age but more common at the age of 3 to 6 months.

2.2.3. Agent related risk factors

Many serotypes of *Salmonella* including S. Enteritidis contain large serotype specific plasmids which encode a range of properties, including virulence factors and are hence referred to as virulence plasmids. Virulence plasmids are one of several *Salmonella* virulence determinants involved in survival and growth in the host cells (Finlay and Falkow, 1988). Galan and Curtis (1989) first characterized the *Salmonella* invasion gene *invA*, the first genes in an operon which is thought to trigger S. Typhimurium in cultured epithelial cells. They further indicated that virulence factors produced by *Salmonella* are responsible for enteric and systemic clinical signs and lesions of salmonellosis.

Barrow (1997) indicated that *Salmonella* is able to invade eukaryotic cells and produce diarrhea. Furthermore, in poultry *Salmonella* can localize in the reproductive tract, alimentary tract or other tissue in the absence of disease which result in entry of these organism into the human food chain. The lipopolisaccharide (LPS) of the O-antigens is associated with the invasiveness and bacterial resistance that has been found towards several antimicrobials like neomycin, tetracycline and some sulfonamides (Old, 1989; Seifert, 1996). The invasion of S. Enteritidis into various organs of chickens and reduction of egg production in hens were reported (Gast and Beard, 1993).

2.2.4. Environmental factors

Most of the members of the *Salmonella* group are killed by exposure to a temperature of 55°C for about 1 hour, or for 15-20 minutes (Old, 1989). It has been found that S. Enteritidis in poultry feed declines with increased time of exposure to heat (Himathongkham *et al*, 1996). Factors including water, rodents, dust, service personnel, staff, vehicles such as feed, trucks and soil stand a high risk of contamination (Swart, 1995). Litter and water are considered to be most useful materials to assess the extent of contamination in the environment. Environmental stress and various infectious diseases were factors which influenced *Salmonella* infections in poultry (Lahellec and Colin, 1985; Read, *et al.*, 1994).

2.3. Poultry salmonellosis

2.3.1. Salmonellosis with host adapted serovars

Avian salmonellosis is divided into three diseases: Pullorum disease (S. Pullorum infection), fowl typhoid (S. Gallinarum infection) and paratyphoid infection (non-adapted *Salmonellae*, including S. Arizonae). It has been found that *Salmonella* species can cause illness in human. Pullorum disease is primarily a clinical disease of the first few weeks of life in chickens, turkeys, game birds, and waterfowl. The most common symptoms observed in *Salmonella* infection are anorexia, diarrhea, pasting of vent, stunted growth, unthriftiness, poor feathering and pale shrunken comb and wattles (Herenda *et al.*, 1994).

Fowl typhoid can produce clinical disease in poultry at any age. Herenda *et al.* (1994) reported that fowl typhoid resulted in enlarged, bronzed liver and spleen, especially in turkeys. There is also enlarged kidneys, pale cadaver and inflammation of intestines. Fowl typhoid produce high morbidity and mortality during the first few weeks of life of any avian species. The infected birds show symptoms that include drooping wings, shivering and huddling near a heat source, muscular incoordination and trembling (Chauhan, 1993). Histopathologic lesions include fibrinopurulent perihepatitis and pericarditis, focal fibrinoid, lymphocytic inflammation, small granuloma and serositis of the pericardium, the pleuroperitonium, and the serosa of the intestinal tract and mesentery (Mallinson and Snoeyenbos, 1989).

2.3.2. S. Enteritidis and S. Typhimurium infection in poultry

S. Enteritidis and S. Typhimurium are some of the paratyphoid organisms producing endotoxin responsible for their pathogenic effects and are mostly isolated among species of *Salmonella*. They are non-host adapted species. Some strains of S. Enteritidis may be particularly pathogenic to chickens and humans (Mallinson and Snoeyenbos, 1989). They are

often localized in the intestine or gall bladder of carrier birds and then eliminated with the faeces and thus contaminate egg shells, feed and water. Infection of young chicks occurs primarily by faecal contamination of shell with paratyphoid organisms and subsequent penetration into eggs (Arnall and Keymer, 1975).

S. Enteritidis is at present, a clinically prevalent serotype in many countries due to consumption of eggs or egg-containing food contaminated with *Salmonella* as a consequence of systemic infection of layer chickens (Julian, 1996).

S. Typhimurium is a very common pathogen of rodents. It can be transmitted to cage and aviary birds by contamination of feeding-stuffs with the faeces of wild birds or by rodents. S. Typhimurium can be isolated in organs rich in reticuloendothelial tissues (such as liver, spleen) apart from yolk sac and caecal (Petrak, 1982).

2.4. Public Health Significance

The risk of human infection due to *Salmonella* has probably increased. Factors most responsible are: utilization of animal by-products and waste in animal feed in developing countries and intensive animal production systems mostly in developed countries (Schwabe, 1984). In Germany, like in many other countries, salmonellosis is recognized as a major cause of acute human gastroenteritis (Kaesbohrer *et al.*, 1993). In Ethiopia (Abera, personal communication), human gastroenteritis and typhoid are some of the major problems which are suspected to be associated with *Salmonella* infection though sources of infection have not been determined. Factors assumed to be involved are water, hygiene, eating habits or any others.

Salmonellosis in human occurs after consumption of contaminated food with *Salmonella*. In most cases salmonellosis in human is influenced by factors such as age, predisposing diseases, *Salmonella* serovar involved and the infectious dose. The incubation period in human is about 6 to 72 hours, and the main symptoms are diarrhea, abdominal pain, mild fever, nausea and vomiting, anorexia, headaches, and malaise. The infectious dose is said to be from 100 to 1.000 bacteria which may cause illness in young children, and in persons who are immunocompromised (Notermans *et al.*, 1996). S. Enteritidis infection can also occur concurrently with immunodeficiency virus (HIV) infection and is one of the common complications of acquired immunodeficiency syndromes (Qin *et al.*, 1995)

Salmonellosis (with the exception of typhoid) is by far the leading cause of food-borne disease in the US with 68 outbreaks, and 6250 cases between 1983 and 1987, including $4.0 billions in economic losses annually (Zeider, 1996). The health hazard, particularly, associated with salmonellosis in poultry, in Malaysia has been recognized for many years but the true incidence of *Salmonella* is relatively unknown (Rusul *et al.*, 1996). It is possible that the increase in *Salmonella* isolations from humans can be attributed to the concurrent increase in the consumption of poultry since poultry is known to be the major source of *Salmonella* worldwide. Bailey and Cox (1996) indicated that though a few strains of *Salmonella* are found in the majority of cases of human illness, all strains are generally considered to have the ability to cause illness in human and are considered equally serious.

2.5. Distribution of *Salmonella* serotypes

2.5.1. Worldwide

Salmonella has a worldwide distribution but the importance of *Salmonella* species differ geographically. Biberstein and Zee (1990) indicated that S. Gallinarum / Pullorum are rare in United States (US). Ziprin *et al.* (1992) proved that incidence in salmonellosis was predominantly due to S. Typhimurium in United States and in Europe. However, the shift towards a predominance of S. Enteritidis was observed in almost all European countries (FAO/WHO, 1992). In Germany 1989 to 1992 records demonstrate that S. Enteritidis is the most predominant serovar followed by S. Typhimurium among all the existing serotypes. From 1990 to 1992 in many other European countries, the predominant serotypes were S. Enteritidis followed by S. Typhimurium (WHO, 1992). S. Uganda, as a new and highly pathogenic organism causing food poisoning, has been isolated in human associated with the consumption of contaminated food in Italy (Ricci *et al.*, 1990). S. Anatum was isolated from raw chicken carcasses in Venezuela (Rengel and Mendoza, 1984).

2.5.2. Africa

Several serotypes of *Salmonella* have been reported in Africa. Swart (1995) reported outbreaks of S. Enteritidis in South Africa, in 1991/1995, in poultry industry and since then strict control measures have been practiced. The presence of wide range of *Salmonella* serotypes in slaughter houses has attracted much attention in recent years in various parts of the world. In Botswana among the most common *Salmonella* serotypes isolated in the slaughter house were S. Anatum isolated from the neck, carcass meal, blood meal and liver and S. Enteritidis isolated from unwashed carcass swabs (Ndzinge *et al.*, 1982).

According to Hummel (1979), in Tanzania, among 436 *Salmonella* strains from different species including poultry, the most prevailing were S. Typhimurium, S. Enteritidis, S. Gallinarum and S. Anatum. The same serotypes are common in Nigeria (Osiyemi 1975), whereas in Ghana S. Typhimurium is the most commonly isolated serovar (Boachie, 1983).

2.5.3. Ethiopia

Unpublished farm report indicated that high mortality in chickens occur due to *Salmonella* in one of the largest commercial layer farm since 1985. This *Salmonella* was assumed to be S. Pullorum or S. Gallinarum because bacteriological confirmation is lacking. Table 2 indicates the high mortality due to *Salmonella* infection and drug expenditure in layers since 1985 to 1992. Teshome and Mebratu (1988) have confirmed the presence of S. Pullorum in 1988 in one broiler farm in Ethiopia. The whole range of the status of *Salmonella* serotypes existing recently is unknown. As new *Salmonella* species infecting poultry are reported worldwide, the existence of several *Salmonella* serotypes are expected in Ethiopia.

Table 2: Relative importance of mortality due to *Salmonella* and drug expenditure from 1985 to 1992 in layer flock in Debre Zeit, Ethiopia. (Dembi farm records).

Year	Flock size	Total mortality	Mortality due to *Salmonella*	Drug expenditure on *Salmonella* (%)
1985-86	2384	847	647	15%
1986-87	136138	8606	204	4%
1987-88	107024	32087	12592	44%
1988-89	173617	56274	24456	52%
1989-90	152973	43917	23242	68%
1990-91	248814	97687	14833	4%
1991-92	31875	5784	2559	47%

Source= Dr. Mohamed Nasser.

Out of the total expenditure of drugs on diseases on this farm, *Salmonella* seem to have a considerable contribution. There was even some increase on drug expenditure from 1988-89 with the highest peak of 68%. in 1989-90

2.6. Prevention and control of salmonellosis

Salmonella infections represent a multifactorial syndrome involving various ecological conditions and routes. Social and cultural behavior of the consumer has a great impact on the severity of food-borne diseases. Control procedures at farm level should go beyond the scope of the merely microbiological traditions (Noordhuizen *et al.*, 1997).
Watner-Toews (1996) indicated the importance of ecological studies that aim at niche engineering which comprises of social, cultural and ecological issues. These studies enable us to know more about the agent factor and its behavior under different conditions

It is generally believed that *Salmonella* are such ubiquitous organisms that total eradication from poultry is virtually impossible and the best that we can hope for is a reduced incidence. Now, the pressure is not only to reduce *Salmonella* contamination, but to completely eliminate some serovars (Anonymus, Poultry International, 1989). The European Council Directive 92/117/EEC prescribes monitoring of poultry breeding flocks and the eradication of flocks contaminated with S. Enteritidis or S. Typhimurium. It is believed that so-called top-down approach is essential in eradication of S. Enteritidis and S. Typhimurium in poultry production chains (Notermans *et al.*, 1996). Major areas of *Salmonella* monitoring in poultry premises are breeding flocks, commercial flocks, hatcheries, restocked animals and breeding eggs (Anonymus, Directeur Dieregesontheid, 1993; Swart, 1995).

Control can be exercised by improved clean egg production and reduction of hatchery transmission mainly by shell contamination (WHO, 1993; Bailey and Cox, 1996). An increase in the number of egg collections from nest boxes per day can effect considerably the contamination rate in eggs that are removed from contact with contaminated faeces in the nest boxes before they have cooled. Collections as often as three times per day are recommended; eggs should be stored in vermin-proof room kept solely for that purpose and maintained at a temperature between 9°C and 13°C and at a humidity of 75%. Nest-box litter must be kept clean and dry and renewed frequently; wood shaving is preferable to straw or hay. Dirty cracked floor eggs should not be incubated (Gordon, 1976).

Salmonella is fairly easily destroyed by most disinfectants and by heat (60°C for 15 minutes), but can remain viable in the environment for lengthy periods, up to 5 weeks in old litter; after an outbreak litter is best dealt with by incineration (Gordon, 1976). Hygienic premises, cleanliness, provision of non-contaminated feed and drinking water as well as appropriate feeding are important prerequisites for the prevention of salmonellosis. Infection of young animals caused by *Salmonellae* are best prevented by type specific active immunization of the mothers with formalin adsorbed vaccines which has to be carried out in time before parturition (Seifert, 1996).

Application of biosecurity steps at all levels of the poultry production system are required. When considering biosecurity measures, the areas of attention include staff and other personnel, equipment such as trucks and other services, feed and water, fly and vermin control especially rodents, cleaning and disinfection procedures, the monitoring of the microbiological quality of water, a monitoring of the full range of poultry production systems and of poultry products, the use of vaccines in grand parent and parent flocks and the strategic use of certain anti-microbial alone or in conjunction with vaccines (Jansen, 1990; Swart, 1995).

Lee *et al.* (1994) showed that disinfection by formaldehyde at hatchery resulted in least embryo deaths of 5% and 95% of the highest chicks survival rate from eggs experimentally infected by S. Pullorum. Notermans *et al.* (1996) recommended the use of competitive exclusion immediately post-hatch to reduce colonization with *Salmonella* of the intestinal tract of chicken. In order to formulate effective preventive control strategies and to understand the epidemiology, information on the prevalence of *Salmonella* in poultry is indeed necessary (Rusul *et al.*, 1996).

Sanitation of the air that circulate in the hatching cabinets and thorough cleaning up of the hatchery are best methods to eliminate *Salmonella* contamination (Bailey, 1993). Swart (1995) indicated the use of vaccination program combining attenuated live S. Typhimurium vaccine with a follow up by inactivated oil based S. Enteritidis vaccine by many countries. Vaccination and competitive exclusion are used increasingly. *Salmonella* has always been recognized as an organism which can enter flocks from a number of sources and control must be exerted at several levels (Barrow, 1997).

3. MATERIALS AND METHODS

3.1. Description of Study Area and Population

3.1.1. Study area

The study was conducted in Debre Zeit which is located about 45 km south-east of Addis Ababa in the Ethiopian highlands. The altitude is about 1850m above sea level. The soils and climate are similar to those in many highland areas in Africa. The main rain season extends from June to September with an average rainfall of 866 mm (of which 84% of rain is expected). There is 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 the rainy months and 8-10 hours for the rest of

the year (ILCA, 1988). The field research commenced in May and extended up to September, 1997. The map of the study area is shown on Appendix 5.

3.1.2. Study population

Eight farms with twenty flocks of chickens were selected. The farms were conventionally designated as A, B, C, D, E, F, G and H and categorized according to ownership and size:
a) Large State Commercial farms,
b) Large Private Farms and
c) Small Private Farms. Table 3 shows the breakdown.

Table 3: Types of farms and their corresponding flocks included in the cross-sectional study

Farm/flock	Sub-division	Type	Number	House mgt.	Age (days)
A	A/1	Layers	16000	Cage	471
A	A/2	Layers	8000	Cage	478
A	A/3	Layers	15000	Cage	501
A	A/4	Growers	2000	Litter	128
A	A/5	Growers	6000	Litter	89- 101
A	A/6	Layers	6354	Cage	188
A	A/7	Layer breeders	1447	Litter	485
A	A/8	Growers	1651	Litter	175
B	B/1	Broiler	700	Litter	69
B	B/2	Broilers	2934	Litter	49
C	C/1	Broilers	250	Litter	42
D	D/1	Broiler breeders	1080	Litter	140
D	D/2	Broilers	4000	Litter	6
D	D/3	layers	1180	Litter	12
E	E/1	Broiler breeders	1070	Litter	308
F	F/1	Layers	1000	Litter	6
G	G/1	Layer breeders	95	Litter	360
H	H/1	Layers	4654	Cage	315,539 714
H	H/2	Pullets	1300	Litter	77
H	H/3	Male growers	1300	Litter	77

mgt= Management

a) Large State Commercial Farms

In this group two farms designated as A and B were included. Both farms are owned and operated by the state.

1) Farm A consists of one of the largest commercial layer farms in the country. This farm has a total of seventeen houses for rearing and production. There is also one hatchery with a capacity of 1,700,000 eggs per annum installed but currently not operational. Eight of the houses are for caged birds and sized between 88m x 12m with a capacity of 18,240 birds.

Two of these houses are of the same capacity but sized 90m x 14m. Nine houses are for rearing. Six of these houses are of the capacity of 15,000 birds with the size of 88m x 12m. The rest has the capacity of 9,000 birds with 50m x 12m. At the time of the research work this farm had eight flocks of chickens with a total of 9,651 birds in the rearing and 46,801 in the production unit.

The building system in this farm consists of the following: foundation is of stones, concrete floors, clay walls, asbestos and iron sheet roofs, doors are of iron sheets. All layers in production are kept in cages. There are 4 rows of cages per house, 4 levels per row per house, and 4 birds per cage. The deep litter system is practiced in all rearing birds.

2) Farm B is a state farm for broilers. This farm has twelve houses. Only two houses were stocked at the time of the study. The foundation of the houses are of stones, floors are of concrete, walls of clay, roofs of asbestos and doors are of iron sheets. Deep litter system made up of *Ergarostis abyssinca* (teff) straws is used. The size of the houses range between 88m x 12.

b) Large Scale Private Farms: There are three farms under this category.

1) Farm D has three flocks (D/1, D/2, D/3). The size of the house for flock D/1 is 13m length x 15m width. Buildings and keeping systems are as above. The size of the houses of the last two flocks are 15m x 6m.

2) Farm E has only one flock, (flock E/1). Building constructions are the same as of the broilers as mentioned earlier. The size of the house is 11m x 12m. There is also a hatchery in operation in this farm with the hatching capacity of 15.000 eggs.

3) Farm H has three flocks (two in rearing H/2 and H3" and H/1 in production). The foundation of the houses are of mud block. The walls and floors are as the above. Roofs are made up of iron sheets for rearing birds and asbestos for layers. Doors are of wood for layers and metal for rearing birds. This farm has a total of three houses sized 20m x 7m. Birds in production are kept in cages and deep litter system for rearing. There is one house for birds in production. This house has 3 rows of cages, 116 cages and 4 to 5 birds per cage.

c) Small Scale Private Farms: This category consists of three farms.

1) In farm C, walls and floors are of mud and thatched roofs. The door is made up of wire mesh. The size of the house is 4m x 3m. Deep litter system is used like in the other farms.

2) Farm G is a layer parent stock. The size of the house is 5m x 3m. The foundation is of stones, walls of mud and roofs are of iron sheets, the door is of iron mash. The keeping system is deep litter system.

3) Farm F is for layer chicks. The size of the house is 10m x 7m. Deep litter system is practiced.

3.2. Management

- **Routine practices**: Depending on type of farm and ownership either state or private, or layer, broiler, breeder stock management differs. General inspection of chicks was done twice daily except in breeder flocks which was done thrice a day. The recording system was very poor in most farms. Eggs are collected manually twice a day. The deep litter system is practiced by all farms and the material for the litter straw is found locally.

- **Feeding**: Locally produced feed by local feed mill other than the poultry producers is used in all farms. The feeding system is either automatic or manual in different farms. Farm A, B and H is automatic.

- **Water**: All farms use spring or ground water. There are farms which use automatic drinkers (Farm A, B and H) and in others it is done manually.

3.3. Study design

The study consisted of historical (retrospective), cross-sectional and longitudinal (prospective) parts. Historical data which were only available in farm A were collected. Eight farms with a total of 20 flocks were included in the cross-sectional part of the study. Questionnaire was conducted in all farms. Farm E was selected for the longitudinal study since it contained positive reactors to fowl typhoid and also S. Enteritidis was isolated. Eggs from this flock were followed to the hatchery. Two farms were supplied with day old chicks from the same hatchery. These chicks were followed up until the end of the rearing period. Appendix 1 indicates the layout of the study design.

3.3.1. Retrospective data

Available retrospective data on flock size, age, egg production, total mortality and mortality due to several causes including salmonellosis from 1992 to 1997 were collected and analyzed.

3.3.2. Questionnaire survey

The questionnaire was designed to assist in getting more information on factors assumed to contribute to the prevalence of *Salmonella* in poultry. This questionnaire focused on the poultry disease history on each farm, biosecurity measures on the farm, flock health status during sampling and also on productivity performance mainly on husbandry of the animals (birds). This questionnaire was conducted in an interview form. According to Pellaz (1996) a modified scoring method was applied in order to be able to classify or rank the farms based on the questionnaire response and also on personal observation. Points were given individually by farm and also grouped according to ownership (state or private), then added up: the greater the points scored the more exposed to risk factors. By this scoring method we could at least reach an estimation of the status of the farm under different management schedules, i.e. large state, large private and small private farms.

The responses were noted 0 or 1. To be able to arrange the points, the class interval (w) was calculated according to Daniel (1991) whereby $w = (R/k)$, R = range and k = number of class interval. The class intervals were 5 in total: very good, good, satisfactory, bad and very bad. The variables (questions) were classified into two: yes versus no (Henken *et al.*, 1991). The

variables were again grouped into three categories: biosecurity measures, hygiene and health status and management. Biosecurity measures are those factors that can horizontally transmit *Salmonella* like rats, equipment, people etc. Only those questions that were common to all farms were used for scoring between the farms, the rest of the questions are indicated in Appendix 2. Some of the questions were derived from the recommendations made by Henriksson (1992) in his publication on building system for broilers and layers and also from the research conducted by Henken *et al* (1991), on the questionnaire analysis applying quantitative epidemiological methods. Swart (1995) indicated the importance of biosecurity measures for the control of S. Enteritidis. These measures were regarded in this questionnaire construction.

3.3.3. Cross-sectional study

Eight farms with a total of 20 flocks of chicken were randomly selected and designated as farm A, B, C, D, E, F and H (Table 3). These farms included two layer commercial farms (A and H), two broiler farms (B and C), two broiler breeder farms (D and E) plus other flocks in production, one parent layer farm (G) and one layer chicks farm (F). Testing of fowl typhoid in seventeen flocks from these farms (A/1, A/2, A/3, A/4, A/5, A/6, A/7, A/8, B/1, B/2, C/1, D/1, E/1, G/1, H/1, H/2, H/3) was conducted. Flock D/2, D/3 and F/1 were not included in this test because they consisted of young chicks below two weeks of age and they are immunologically immature. No serological response to somatic antigens can be expected (WHO, 1994).

The test was conducted on the farms. Agglutination of blood samples with stained S. Gallinarum antigen was used (WHO, 1994). 30 samples were tested per flock in order to be 95% certain of detecting at least one positive animal if the disease is present at 10% (Cannon and Roe, 1982). A flock is declared positive if at least one sample is positive. Bacteriological examination was done to isolate *Salmonella* and to identify serotypes involved. Ten liver samples from dead chickens were pooled as one sample. The same was done in caecal samples. Other samples that were collected and processed culturally were litter, water, feed, surroundings, dust etc as indicated in section 3.4.2.1.

3.3.4. Longitudinal study

Farm E was selected for longitudinal study. The farm consisted of broiler parent stock. One batch of eggs from this flock was monitored from the production of eggs, hatchery, distribution of day old chicks in two different farms to age of slaughter. The sampling regime included the hatchery, the stable before the arrival of day old chicks at the two selected farms, at 7 days old, at 21, at 35 and at 49 days old. Each farmer was supplied with 300 day old chicks. A detailed sampling strategy is indicated in section 3.4.3.3.

At the arrival of the chicks at both farms, three faecal samples from the transport boxes were taken of which 100 samples were pooled to represent one sample. At seven days old both farms were sampled. Six litter samples, feed, water, swabs from the surrounding of the pens, swabs from the inside of the pen were taken. Study design is shown in Appendix 1. This strategy was adopted from Kaesbohrer and Blaha (1997) on their study on field experiences with monitoring and reducing *Salmonella* in poultry meat production chains and from the recommendations by WHO (1994) on monitoring of S. Enteritidis in flocks.

3.4. Collection, transportation and storage of samples

3.4.1 Sampling procedures

All samples were collected under aseptic conditions to prevent contamination or cross-contamination. This included the use of sterile sampling materials (i.e. swabs, applicators, bags, etc) and disposable gloves. The samples were packed in sterile plastic bags and transported directly in a cooler box with ice to the Faculty of Veterinary Medicine Microbiology Laboratory and kept in the refrigerator at (2-4°C) until processed. Dead birds were also transported under cold chain. All samples were labeled and accompanied by necessary identifying information. This included the date of sampling, the type of sample and the specific farm, flock, the hatchery, etc.

3.4.2. Types and methods of collection sample

The standard methods outlined by WHO (1994) and the strategy established by Käsbohrer and Blaha (1997) were followed and listed as follows.

3.4.2.1. Sampling scheme in the cross sectional study

a) Blood sampling

Blood samples were taken from seventeen flocks, A/1, A/2, A/3, A/4, A/5, A/6, A/7, A/8, B/1, B/2, C/1, D/1, E/1, G/1, H/1, H/2 and H/3. 30 chickens from each flock were tested. A drop of blood was withdrawn from the wing vein of a bird by pricking with a suitable needle. The blood was placed on a clean slide. A polyvalent crystal violet stained S. Pullorum antigen was added to the blood and mixed well. The slide was rocked for 2 minutes after which the test was read to see if there was agglutination or not. The flock was declared infected if at least one animal reacted positively to this test (WHO, 1994).

(b) Environmental samples

(i) Drag Swabs: The samples were collected within the houses and all flocks were sampled. 10x10 cm surgical swabs per sample were autoclaved and placed in a sterile container. When opened, the swabs were dropped into a sterile beaker of Buffered Peptone Water (BPW) to moisten them before dragging them up and down the pen surface. These were placed into BPW for transport to the laboratory. This brings a total of 20 samples from all flocks.

(ii) Foot Swabs: A total of 5 samples from all caged flocks were sampled (A/1, A/2, A/3, A/6 and H/1). A large surgical swab was sterilized and fasten under a shoe of an attendant and worn during house activities such as egg collecting or inspection within the house. At the end of this period, the swab was removed and placed into the jar containing 225ml BPW and dispatched to the laboratory for culture.

(iii) Dust: Farms A/1, A/2, A/3, A/6 and H/1 were sampled. Approximately 2.5g samples were collected at ten different sites in the house and bulked to give 25g per sample. A total of 5 samples were collected. This pooled sample was placed in a sterile BPW, tightly capped and transported.

(vi) Floor sweepings were collected using an autoclave brush and bulked to give 25g. These samples were collected from A/1, A/2, A/3, A/6 and H/1.

(c) Others:

(i) Litter samples: Litter was collected from ten different sites within the house and then pooled to make one sample of which six of these were collected from each flock selected and placed in sterile sampling plastic bags (60 sample size per flock). These flocks were A/4, A/5, A/7, A/8, B/1, B/2, C/1, D/1, D/2, D/3, E/1, F/1, G/1, H/2 and H/3. Each sample was mixed well and 25g was weighed to make one sample. Sterile gloves were used for collecting and samples were placed in a cooler box with ice to the laboratory.

(ii) Faecal samples: All caged flocks were sampled. Ten droppings from ten different sites within the house were pooled to represent one sample (60 sample sites per house). Six faecal samples were collected. Sterile gloves and plastic bags were also used. Five flocks were sampled, flocks A/1, A/2 A/3, A/6, H/1. All caged flocks were selected for faecal sampling.

(iii) Liver samples: Flocks A/1, A/2, A/3, A/4, A/5, A/6, A/7, A/8, B/1, B/2, D/2, D/3 and F/1 were sampled from the dead and sick birds, up to ten livers were pooled together to make one sample from each flock where mortality occurred during sampling.

(iv) Caecal samples: From postmortem samples, up to ten caecal samples from the dead and sick birds per flock were collected and macerated in BPW. Caecal samples were collected from all farms which were sampled for liver.

(v) Feed samples: Feed samples from the feed bins were collected from all twenty flocks. A 25g of feed was collected per flock in a sterile plastic bag.

(vi) Water: One liter of water from the main source per farm was collected in a sterile container. A total of eight water samples were collected (one sample per farm).

(vii) Swab from egg sorting area: Flocks A/1, A/2 A/3, A/6, A/7, E/1 and H/1 were sampled. Ten sterile surgical swabs were used and represented one sample from each layer flock and placed in sterile plastic bags.

(viii) Swabs from the surroundings of the farm: All eight farms were sampled and ten sterile surgical swabs as one sample were used as above per farm. All the surroundings including the equipment were swabbed.

(x) Nest box sample: Ten sterile surgical swabs were used to collect dust from the nest as one sample and placed in a sterile plastic bag from farm A/7, E/1 and G/1.

3.4.3. Sampling for longitudinal study

3.4.3.1. Sampling in the parent stock

As it has been indicated, farm E was the parent stock which was selected for the longitudinal study. Samples like blood, water, feed, drag swabs inside pens, surroundings, nest box and egg sorting area were collected. This means that the same strategy applied in the cross-sectional study was used.

3.4.3.2. Sampling at hatchery

In the hatchery, the following samples were collected:
a) 10 sterile surgical swabs were pooled to make one sample. These were used on the walls and floors of the incubator before the beginning of hatching.

b) 30 dead in shells from the hatchery were collected in a sterile plastic bag and placed in a cooler box to laboratory where they were kept in a refrigerator.

c) Meconium samples were collected from 600 chicks just after hatching and pooled into 6 samples and placed in a sterile container.

d) Ten chicks consisted of culls and early motalities were collected whereby samples of livers from these were taken aseptically and kept in a refrigerator at freezing point.

e) Hatchery surrounding was sampled using 10 sterile swabs.

f) One liter of water was collected in a sterilized container.
All samples followed a cold chain to the laboratory where they were stored in a refrigerator accordingly.

3.4.3.3. Sampling at production level

A strategic sampling before and at the arrival of chicks at the selected farms (farm E1/1 and E1/2) was done and also at 7 days old, 21 days, 35 days old, and finally at 49 days of age i.e. two weeks before slaughter. Table 4 and 5 show the sampling strategy.

Table 4: Strategic sampling before the arrival of chicks up to 7 day old

		Farm					
		E1.1			E1.2		
Samples	No. samples (pooled)	0 d	1 d	7 d	0 d	1 d	7 d
Drag swab	1 (10)	y	y	y	y	y	y
Water	1 liter	y	y	y	y	y	y
Feed	25g	y	y	y	y	y	y
Surroundings	1 (10)	y	y	y	y	y	y
Liver	1 (10)	n	y	y	n	y	y
Litter	6 (25g)	y	y	y	y	y	y

0 d = sampling at the farm before arrival of chicks
1 d = sampling at the arrival of chicks at a day old
7 d = sampling at 7 days old
y = yes where samples were collected.
n = no where samples were not taken because there were no mortalities

Table 5: Strategic sampling frame from 21 to 49 days old

		Farm					
		E1.1			E1.2		
samples	No. samples (pooled)	21.d	35.d	49.d	21.d	35.d	49.d
Drag swab	1 (10)	y	y	y	y	y	y
Water	1 liter	y	y	y	y	y	y
Feed	25g	y	y	y	y	y	y
Surroundings	1 (10)	y	y	y	y	y	y
Liver	1 (10)	y	n	n	y	y	n
Litter	6 (25g)	y	y	y	y	y	y

21 d = sampling at 21 days old
35 d = sampling at 35 days old
49 d = sampling at 49 days old
y = yes where sampling was done
n = no where sampling was not conducted because there were no mortalities

3.5. Microbiological Procedures

3.5.1. Glassware and sterilization

Glassware was first soaked in detergent, washed with the same detergent solution and finally rinsed with distilled water. It was then dried and sterilized in hot oven at 160°C for one hour. Used materials were autoclaved at 121°C for 15 to 20 minutes, cleared from agar and other materials, then soaked in detergent, washed and sterilized in hot oven as above. Types and preparation of media used are shown in Appendix 3.

3.5.2. Test sample preparation

Samples were processed systematically according to their time of arrival in the laboratory. Labeling of samples for laboratory processing was done. Frozen samples (liver and caecal) were thawed before processing by leaving them at room temperature.

3.6. Cultural Procedures

3.6.1. Pre-enrichment

Buffered peptone water was used as pre-enrichment broth.

Litter- From each sample, 25g were weighed and mixed well with 225ml of BPW by using a stomacher 400 (Seward) for 60 seconds at a normal speed and incubated at 37°C for 16 to 20h.

Faecal- Droppings were pooled as one sample and macerated in 225ml of BPW by means of stomacher 400 (Seward) for 60 seconds at a normal speed and incubated at 35°C to 37°C for 16 to 20h.

Dust- 25g of dust were pre-enriched in 225ml of BPW as indicated above at 37°C.

Floor sweepings- 25g of floor sweepings were collected and pre-enriched as above in 225ml of BPW.

Feed- 25g were collected and the procedure was the same as of the samples mentioned earlier.

Water- One liter of water sample was put through a 10x10cm five times folded sterile gauze and the gauze was pre-enriched in 225ml BPW at 37°C for 16 to 20h.

Liver- Up to ten livers from the dead, sick or culls were collected separately using sterile forceps and put into sterile plastic bags containing 50ml BPW. The samples were further macerated with a stomacher 400 (Seward) for 60 seconds at a normal speed and with a sterile cotton butt directly inoculated onto MacConkey agar and incubated at 37°C for 24h.

Caecal- Up to ten samples of the caecal were chopped with sterile scissors and homogenized in a stomacher 400 (Seward) for 60 seconds at a normal speed in 225ml BPW and incubated at 37°C for 16 to 20h.

Meconium samples- 100 samples were pooled and pre-enriched in BPW at 37°C for 16 to 20 h.

Dead in shells- 10 dead in shells were pooled and disinfected with 70% alcohol and opened aseptically on a sterile Petri dish carefully to be able to collect the yolk sac. These 10 yolk sacs were macerated and pre-enriched in 225ml BPW as above.

Swabs from egg sorting area, the surroundings, the nest-boxes and swabs from wall and floors of incubator- Ten sterile swabs were used as one sample for each sample mentioned and pre-enriched in BPW at 37°C for 16 to 20h.

Drag swab- Ten sterile swabs were autoclaved and placed in a sterile container of 225ml BPW. These were dragged up and down the pen surface. They were then pre-enriched in BPW at 37°C for 16 to 20h as one sample.

Foot swab- A large swab which was used to collect samples during activities within the house was placed in BPW and cultivated at 35°C to 37°C for 16 to 20h.

3.6.2. Enrichment in selective liquid media

After 16 - 20h of incubation of samples on pre-enrichment media, 0.1ml of the culture from each sample was aseptically transferred to 10ml of RV and TGB- broths and incubated for 24h at 40°C to 42°C.

3.6.3. Plating on selective media

After the enrichment process, each sample was plated out onto a pre-dried BPLS and XLD agar prepared on large-sized Petri dishes. For inoculation, a loopfull was streaked onto these agar plates and incubated at 37°C for 24h. These plates were examined for the suspected *Salmonella* colonies whereby three to five colonies were selected based on typical characteristics of the colonies. On XLD agar the colonies are the same colour as the culture

medium, translucent and typical with a black center; whereas on BPLS agar the colonies are red surrounded by a bright red zone and the culture medium is red. The single suspected *Salmonella* colonies either from XLD or BPLS for each sample were then streaked on pre-dried nutrient agar plates for each sample in a manner that allowed well isolated colonies to develop. These were then incubated at 37°C for 24h. The suspected colonies were then subjected to a confirmation using standard methods recommended by ISO (1993).

3.7. Biochemical characterization of isolates

The isolates were biochemically differentiated from other Gram-negative genera using reaction on TSI agar slopes, lysine decarboxylase broth and Simmon citrate agar slopes. These were performed in order to increase the likelihood of obtaining suspected *Salmonella* colonies for serological confirmation. Apart from the TSI reaction, negative reactions to both citrate utilization and lysine decarboxylase were discarded.

3.8. Serotyping of isolates

In order to proceed with the serological confirmation elimination of auto-agglutinable strains was done. Isolates were screened for any autoagglutination before the full serotyping was conducted. For this test, a drop of saline solution was placed onto a clean glass slide. A loopful of colony to be tested was mixed thoroughly so as to obtain a homogeneous and turbid suspension. The slide was rocked gently for 30 to 60 seconds. The results were observed against a dark ground, using a magnifying glass. The strain was considered autoagglutinable if the bacteria had clumped into more or less distinct units and was not to be submitted for further tests.

3.8.1. Testing of suspected colonies

The detection of the presence of *Salmonella* "O"-, "H"- and "Vi"- antigens was tested by slide agglutination with the appropriate sera (Sifin, Berlin) from pure colonies grown on TSI agar slants (Buxton and Fraser, 1977). Testing was sequentially conducted as shown in section 3.8.1.1. to 3.8.1.3.

3.8.1.1. Testing with polyvalent I and II antisera (Sifin, Berlin)

A drop of Anti-*Salmonella* I was mixed well with a single colony on a clean slide from TSI slants. It was then gently rocked for about one minute and agglutination observed was characterized by clumping. If there was no agglutination, Anti-*Salmonella* II was used as above to check for agglutination. Isolates that reacted to Polyvalent I or II were transferred to Gassner agar and incubated for 24 hours at 37°C.

3.8.1.2 Testing with group specific B and D antisera

Serotypes that reacted to Polyvalent I and II were tested against Anti-*Salmonella* B and D groups. From the colonies grown on Gassner agar, a single colony was picked and mixed separately with both sera as above. Subculture of a single colony was made in case of positive reaction from both groups (B and D). From isolates that reacted to group D or B a single

colony was transferred to nutrient agar and incubated for 24 hours at 37°C. The isolates were then transported under cold chain to BgVV, Berlin for final confirmation.

3.8.1.3 Serotyping at BgVV, Berlin

For serotyping isolates are 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 as described above. Afterwards, from the same colony, a small amount is used to check for "H"-antigens in the same way. Material from the same colony is transferred to a semi-solid agar plate and incubated overnight. Testing is done in the same way, looking for the second H-phase of *Salmonella*. Results of the first day are confirmed by repeated testing.

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

3.8.1.3. Testing for antimicrobial resistance in BgVV, Berlin

Seventeen antibiotics were tested against the strains detected. These drugs were as follow: Amikacin (AK 30), Ampicillin (AMP 10), Cefuraxolin (CXM 30), Chloramphenicol (C 30), Colistin (CT 10), Enrofloxacin (ENR 5), Furazolidon (FR 100), Gentamycin (CNN 10), Kanamycin (K 30), Nalidixin acid (Na 30), Neomycin (N 10), Polymyxin (PB 300), Streptomycin (S 25), Sulphamethoxazol/Trimethoprim (SXT 25) and Trimethoprim (W 25). 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 give a growth of non-confluenting colonies on the plate. Commercially (OXOID) available platelets containing standardized amounts of antimicrobials were placed on 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 and interpreting these on the basis of the breakdown given in the results.

3.9. Data analysis

All data collected including the historical and questionnaire were stored and analyzed in MS Excel. The descriptive statistics were used to define mortality rates. Analysis of the historical data was done using mortality rates and presented in the form of graphs, tables and figures. Time series data for the period of 5 years were analysed using regression and analysis of variance (ANOVA) to see if there is significant difference of mortality due to salmonellosis between months. Median monthly egg production was also done in order to assess the influence of salmonellosis on egg production. No other historical data were available from any other farm. The 95% confidence interval rate according to Cannon and Roe (1982) was applied in the blood sample testing. The class interval width was calculated in order to determine the scoring according to Daniel (1991). Graphs were drawn using MS Excel to define mortality rates due to *Salmonella* and other diseases. Antimicrobial results were analysed based on the description by AVID (1996).

4. RESULTS

The objective of the study was to detect the presence of different *Salmonella* serotypes with special emphasis on those pathogenic organisms which are a threat to human and also those that cause economic loss, and the description of their major routes of infection.

4.1. Retrospective data

During the conduction of the questionnaire, there was some information which was found to be valuable to include. The history of the farms concerning diseases was looked into. Farm A was the only farm that reported salmonellosis outbreaks every year since 1985 to 1997. Data from other farms were not available. A lot of missing data between some years were observed like flock size, age, egg production, causes of mortalities. We therefore decided to conduct the historical analysis from 1992-97 in farm A. The information that was available from this period included at least the above mentioned data. The interest in this flock was because it is for layers since S. Enteritidis is more associated with layers. The production period starts from July to June in this farm. Therefore the data have been analyzed based on the practical production period.

This historical part was to define the mortality due to *Salmonella* infection and other diseases and observe their effect on egg production from 1992-97. In figure 1 the flock was 455- 429 days old at the beginning of July, 1992 and 761- 978 days old at the end of June 1993.
Figures 1 to 5 show the breakdown of total mortality rates per month in different years.

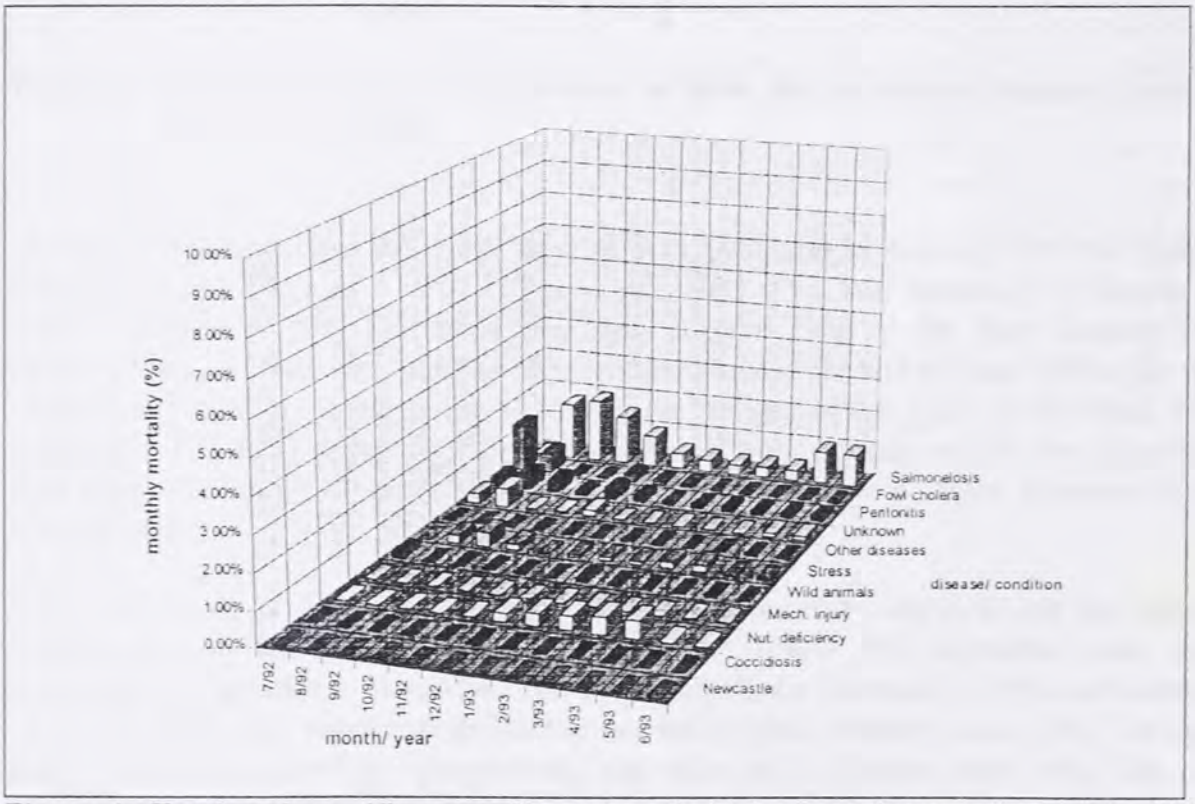


Figure 1: Total and specific mortality rates of chickens at farm A from July 1992 to June, 1993.

Salmonellosis was the major cause of mortality. *Salmonella* outbreaks were observed in almost all months but mortality was highest from mostly August to October. During these months there were mortalities due to some other diseases which occurred like fowl cholera which was registered in the beginning of the production period (July). Peritonitis, nutritional deficiency, stress and some unknown diseases were also observed (figure 1).

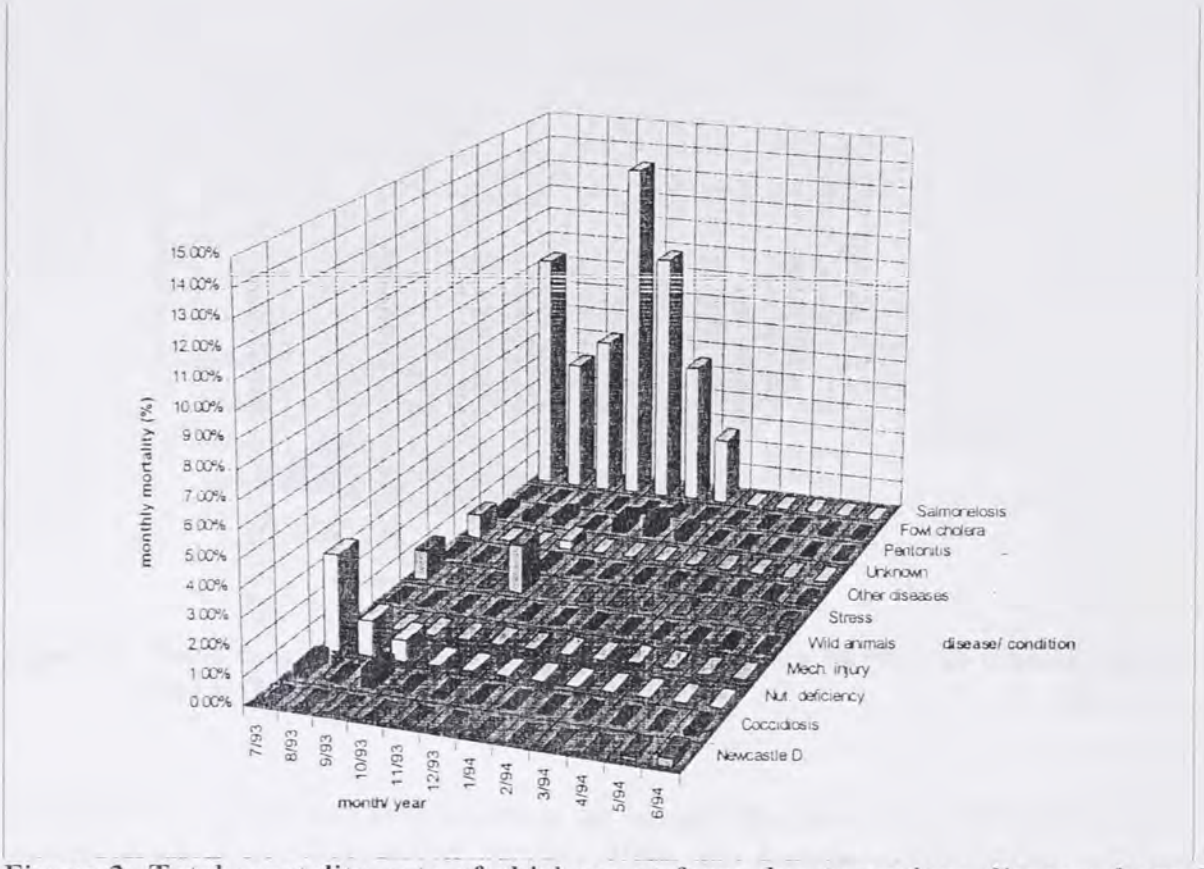


Figure 2: Total mortality rate of chickens at farm due to various diseases from July, 1993 to June, 1994.

The age of the flock was 800-1.008 days in July 1993 and in January 1994 the flock was depopulated at the age of 1.164-1.193 days old. New flock was introduced in March 1994 with 128-165 days old. This flock was only a short time in the farm because to my understanding this flock was transferred to another place at the end of June 1994 at the age of 136-225 and therefore it was not possible to trace the production cycle of this flock. During this time in July 1993 there was 9% mortality due to salmonellosis and 3% due to nutritional deficiency. Mortalities lower than 1% were caused by coccidiosis, stress, unknown diseases and peritonitis.

During the month of August, 5% of mortalities were caused by salmonellosis and about 1% mortalities were due to nutritional deficiency and below 1% mortalities were due to peritonitis. In September mortalities due to salmonellosis increased to 6% and mortalities caused by peritonitis, nutritional deficiency and coccidiosis remained below 1%. The highest peak of mortality caused by salmonellosis was observed in October with 13%. This month was extremely hot and dry. Mortalities attributed to stress and other unknown diseases were observed. During the month of November mortality due to salmonellosis decreased to 9%, and in December 5.3%, and in January 2.5%. During these months there were also mortalities

caused by peritonitis but in a very low percentage. In May to June there were mortalities caused by Newcastle disease and coccidiosis in a very low percentage (figure 2).

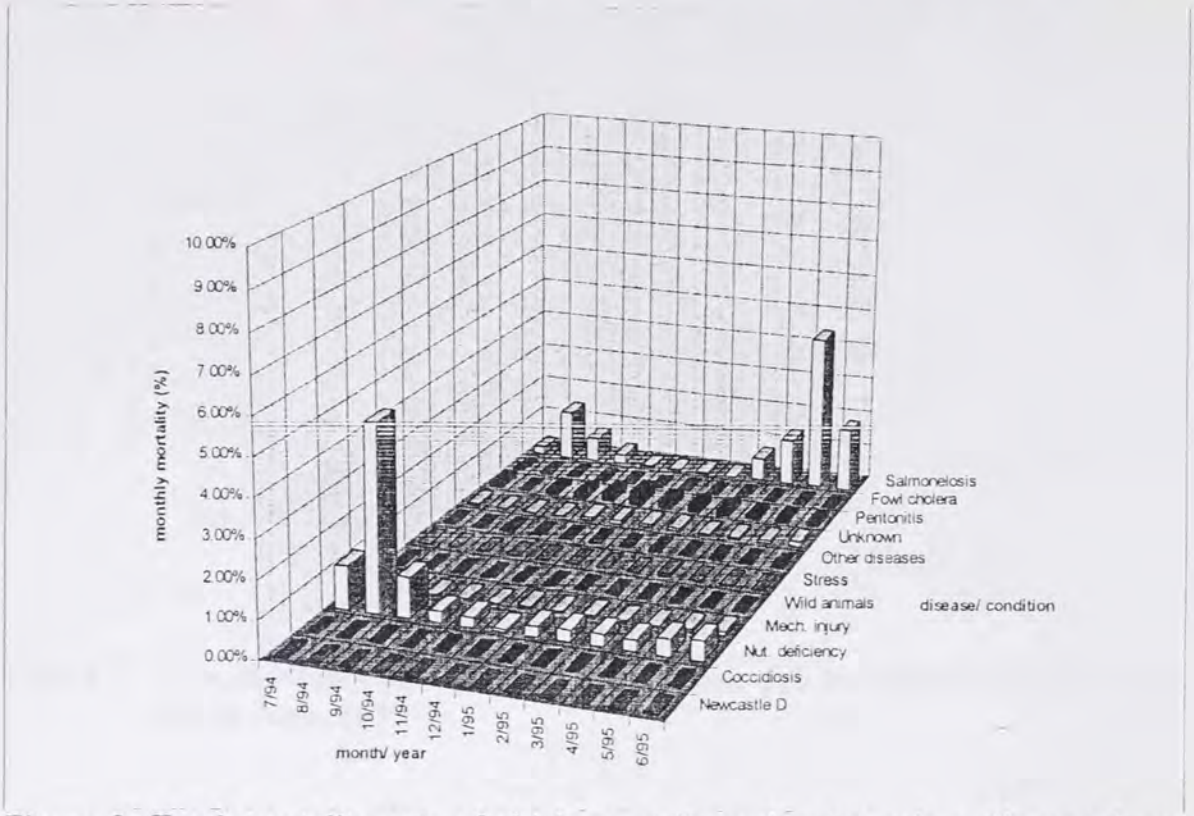


Figure 3: Total mortality rate of chickens at farm due to various diseases from July, 1994 to June, 1995.

New flock was introduced in this farm at the age of 106-260 in July 1994 and in June 1995 the flock was 461-554 days old. In July 1994, the highest mortality due to nutritional deficiency of about 1% was recorded. Salmonellosis and peritonitis mortalities stayed below 0.5% during this month. In August 1994 high mortality was caused by nutritional deficiency which increased to 4%, that was the highest peak observed. Salmonellosis mortalities also increased to 1.3%. In September mortalities caused by nutritional deficiency dropped to almost 1% and also there was a decrease of mortalities due to salmonellosis up to 0.5%. During October mortalities due to salmonellosis and nutritional deficiency remained lower than 0.5% and there were mortalities caused by peritonitis in lower percentage also. From November to February there were no mortalities caused by salmonellosis but they were due to peritonitis, nutritional deficiency, and also caused by unknown diseases. Mortalities caused by mechanical injury was observed in December. From March to June 1995 there were always mortalities caused by nutritional deficiency, peritonitis, and salmonellosis. An episode of salmonellosis started in March and reached a very high mortality rate of 4% in May. This month is just before the rainy season starts (figure 3).

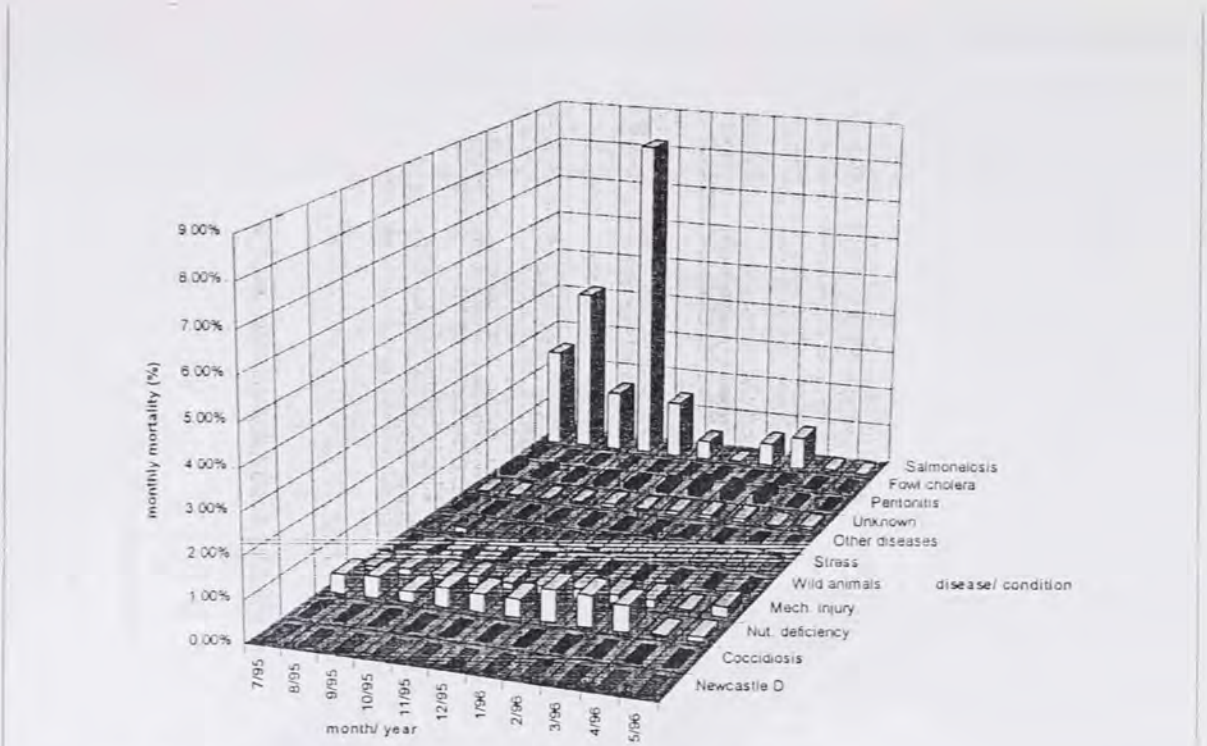


Figure 4: Total mortality rate of chickens at farm due to various diseases from July, 1995 to June, 1996.

The flock begins with the age of 491-584 in July 1995 and in April 1996 the flock was depopulated at the age of 860 days. A new flock was introduced in May 1996 aging 143 days. At the end of the production period in June 1996 new flock aged 173 days arrived. During the month of July 1995 there were 2.3% mortalities due to salmonellosis followed by 0.5% mortalities caused by nutritional deficiency and below 0.5% mortalities due to peritonitis and mechanical injury. In August mortalities attributed to salmonellosis increased to 4% while mortalities due to nutritional deficiency remained at 0.5% and mortalities due to peritonitis, stress and mechanical injury remained lower than 0.5%. In September there was a decrease to 1.3% of mortalities due to salmonellosis and decrease to 0.25% of mortalities due to nutritional deficiency while mortalities caused by peritonitis and mechanical injury remained lower.

During October there was a remarkable increase of mortalities due to salmonellosis of up to 8.2% and increase of 0.5% of mortalities due to nutritional deficiency. Meanwhile mortalities caused by peritonitis, mechanical injury and some unknown diseases were observed in a very low percentage. In November there was a drop of mortalities due to salmonellosis up to 1.4%; meanwhile mortalities caused by peritonitis, nutritional deficiency mechanical injury and some unknown diseases remained the same as in the previous month. In December mortalities due to salmonellosis decreased even more by 0.25% while mortalities caused by nutritional deficiency, peritonitis, mechanical injury remained the same as in the previous month.

During January, 1996 there were increased mortalities due to nutritional deficiency to almost 1% which remained constant in February, 1996. No mortalities due to salmonellosis in the month of January occurred but there were mortalities caused by peritonitis, mechanical injury and some unknown diseases at the same level as in February. In March there were mortalities caused by salmonellosis and nutritional deficiency, mechanical injury, peritonitis and some unknown diseases. In April only mortalities due to mechanical injury and salmonellosis were

observed. In May there were mortalities due to nutritional deficiency and mechanical injury and some unknown diseases (figure 4).

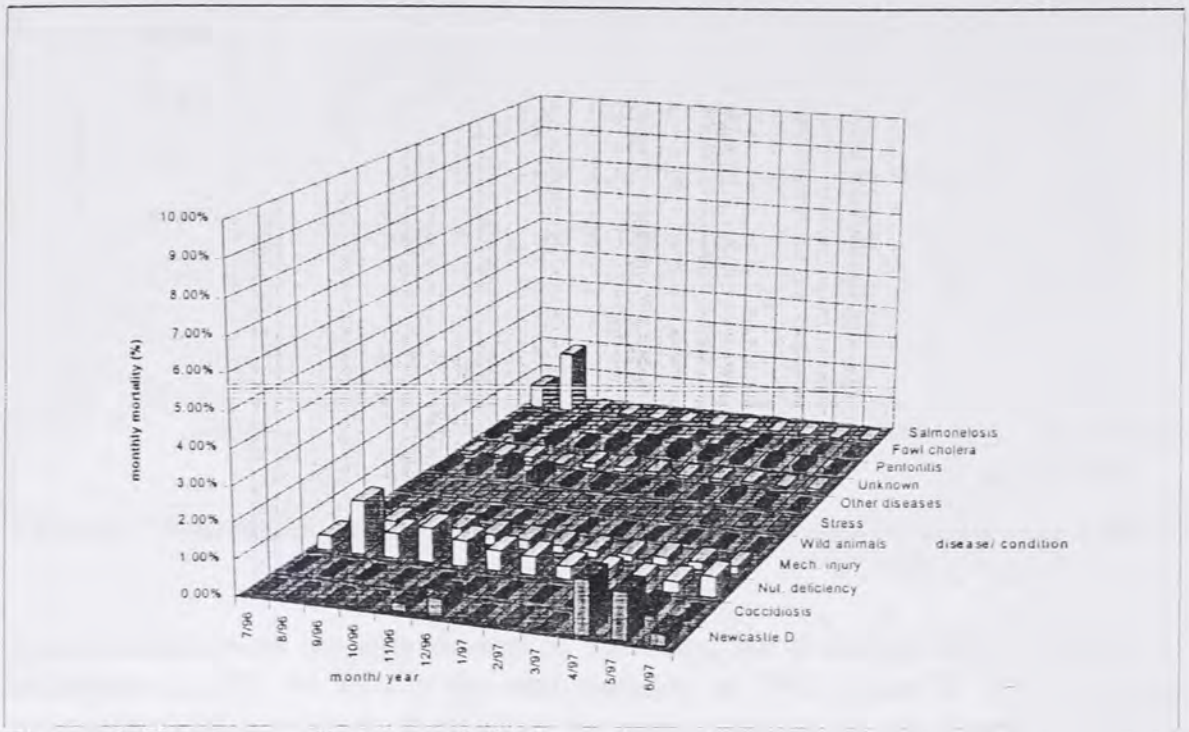


Figure 5: Total mortality rate of chicks at farm due to various diseases from July, 1996 to June, 1997.

In the year 1996-97, cases of mortalities due to *Salmonella* infection were only observed in July and August which are months in the rainy season. There were no *Salmonella* deaths observed until June 1997 but other diseases like Newcastle disease became prominent in November, December, April, May and June. Other mortalities which were observed were due to coccidiosis in May. Peritonitis, mechanical injury and other diseases including the unknown cases were observed almost throughout the year though in a low percentage. During the course of the year there had been remarkable nutritional deficiency in all months with the highest peak in August of about 1.5% (figure 5).

Salmonellosis is not the only cause of mortality in this period of study but it has the highest impact comparing to other causes of mortality. Figure 6 indicates the breakdown of total mortality and mortality assumed to have been caused by salmonellosis during 1992- 97.

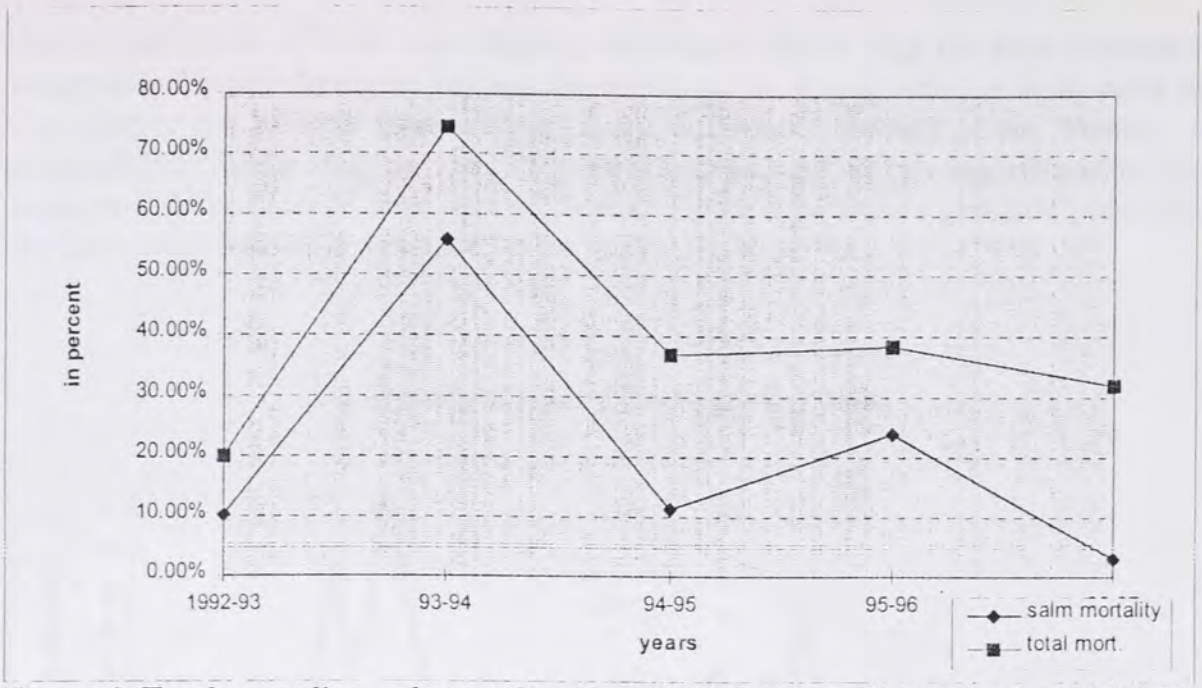


Figure 6: Total mortality and mortality due to salmonellosis from the year 1992- 97

Salmonellosis is not the only disease in this farm, but it showed highest mortality which contributes in 1993-94 56% to the total mortality of 75% (figure 6). This trend continues, however at different levels throughout the period studied in this historical data analysis. Figure 7 shows the salmonellosis outbreak trend in different months from 1992 to 1997.

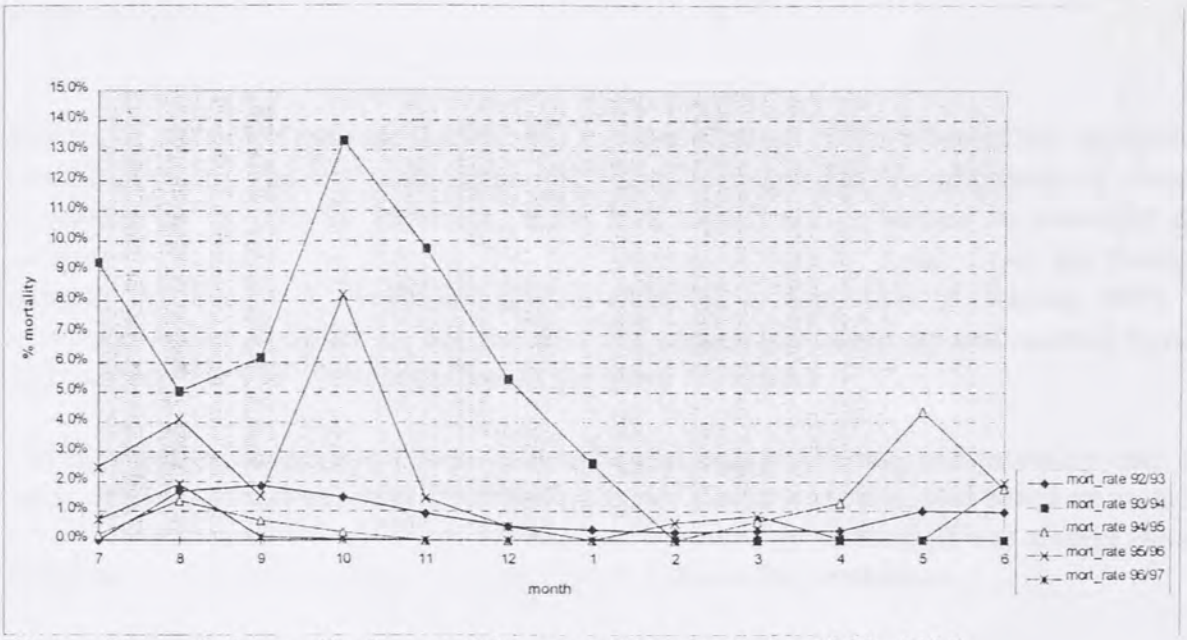


Figure 7: Salmonellosis monthly mortality rate from 1992- 1997

mort. rate= mortality rate

There seems to be a pattern of salmonellosis outbreaks in different months. Between July to September there is some increase of salmonellosis outbreaks in 1992-93, 1994-95, 1995-96 and 1996-97. In 1993-94 a high rate of outbreaks had been observed during July, and it stayed at a high rate compared to other years. Between the months of September and October there is

a sharp increase in 1993-94 and 1995-96. This shows that at least the most outbreaks were observed in October during the hot and dry season and in August which is in the rainy season. Therefore to see whether there are significant differences between certain months, a time series analysis for the study period of 5 years was conducted. Simple regression and ANOVA based on 95% confidence limit were calculated. Figure 8 provides a graphical presentation of the time series data.

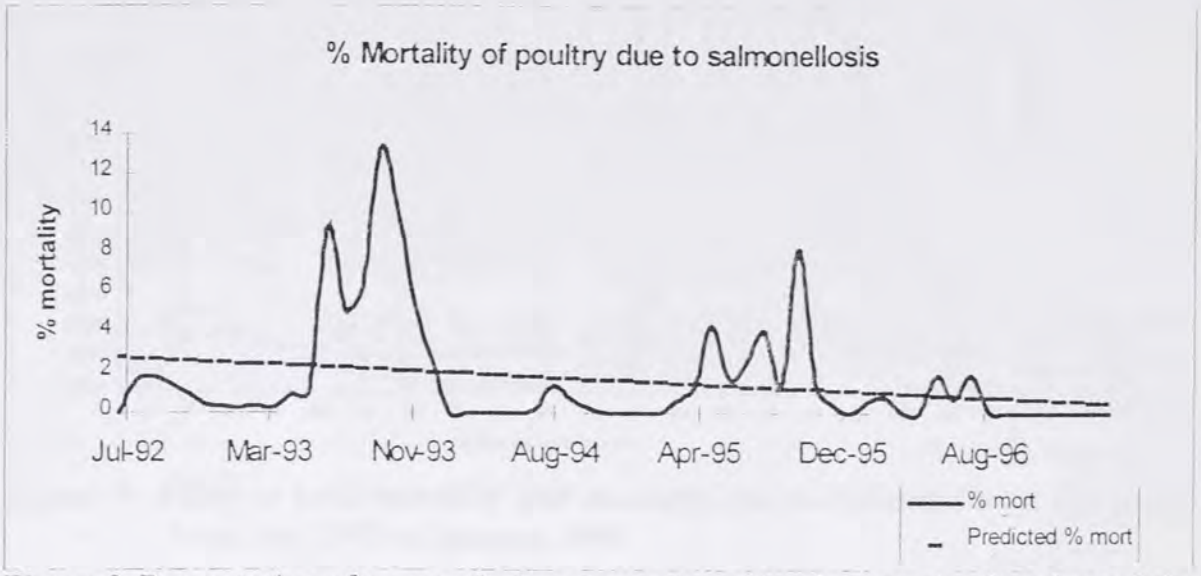


Figure 8: Presentation of a smoothed curve and predicted regression line from July 1992 to June 1997

mort= mortality

Results of this graph indicate that there is a slight increase on the slope of the regression line (predicted value). The regression square (R^2) of 47% means that this percentage of variation is explained by the month. However, there is a significant difference in mortality due to *Salmonella* between the months ($P< 0.05$) using ANOVA. Apart from the increase in mortality in June 1993, the highest peak is observed in September to October, 1993. These months as indicated earlier are hot and dry. The second elevations are seen around April, July and October 1995. In all these outbreaks the curve fluctuates.

During these years there has been comparison of egg production and mortality rate due to salmonellosis or other diseases. The median age of flocks was calculated based on months and also the egg production was given per hen and month. Egg production was always calculated based on the number of surviving birds. Figure 9 shows the breakdown.

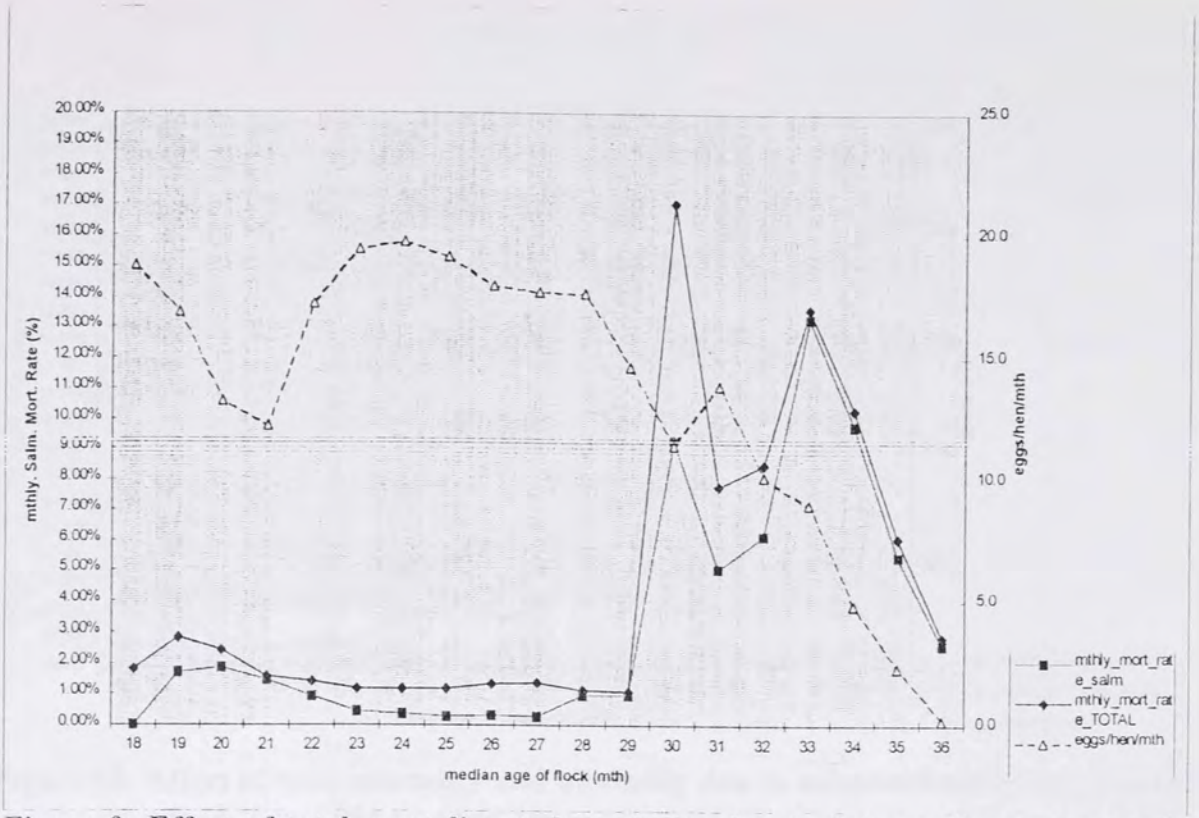


Figure 9: Effect of total mortality and mortality due to Salmonella on egg production from July 1992 to January, 1994

mthly mort. rate salm = Monthly mortality rate due to salmonellosis.
mthly-mort-rate-TOTAL = Total monthly mortality rate due to other causes.

There had been mortalities caused by salmonellosis in all months, but the sharp rise in mortalities due to salmonellosis and other diseases in the 30th month of age led to a substantial drop down on egg production. During this period apart from mortalities due to salmonellosis that contributed almost 50%, other causes of mortalities were due to nutritional deficiency, other diseases like coccidiosis, respiratory diseases and unknown causes (figure 9).

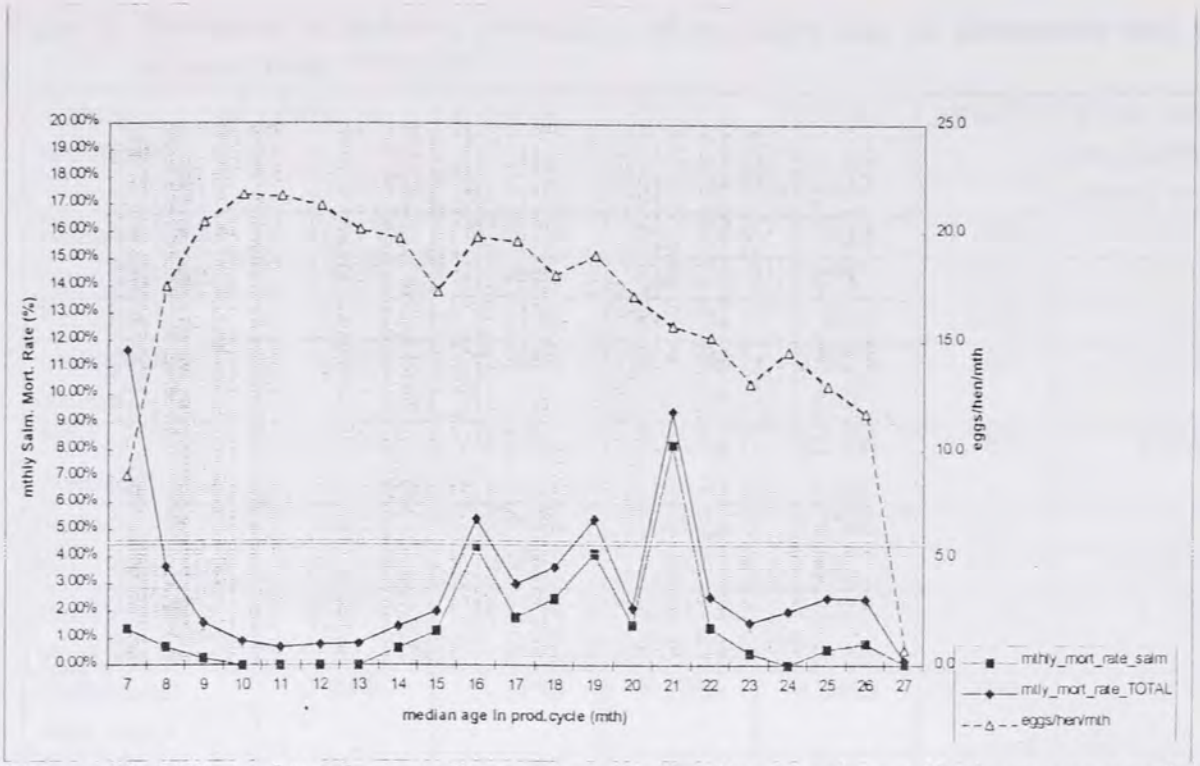


Figure 10: Effect of total mortality and mortality due to salmonellosis on egg production from July 1994 to April 1996

mthly mort rate salm = Monthly mortality due to salmonellosis (as last page)
eggs/hen/mth = Egg production per hen per month.
mth = month.

The production curve is typical but there was a sudden drop of egg production at the end of the cycle. There is an increase of salmonellosis in the 15th month. There was a high mortality due to salmonellosis but this did not reduce the egg production. The reason for the decrease of egg production in the 27th month is not known (figure 10).

These two production cycles show that mortalities due to salmonellosis occurred and increased as the animals were getting older or rather when they are in the last phase of production. There seems to be no year that the flocks were free from *Salmonella* infection but in some years mortalities stayed at a very low percentage. The production cycle 1 (figure 9) shows the effect of *Salmonella* infection to egg production. From the 18th month there was an increase of *Salmonella* infection which affected the egg production. As the infection decreased the egg production was elevated from the 20th month. From the 29th month there was an increase of other diseases and salmonellosis. During this period salmonellosis was not the only cause of mortality but it had the highest impact comparing to other causes of mortality. Table 6 indicates the breakdown of mortality by different causes during 1992-97.

Table 6: Summary of Relative percentage of mortality due to *Salmonella* and other diseases from 1992-1997

Cause of mortality	1992/93	1993/94	1994/95	1995/96	1996/97	Total average mortality over 5 years
Salmonellosis	10.41%	51.57%	10.54%	21.58%	2.79%	19%
Fowl cholera	2.46%	0%	0%	0%	0.03%	0.5%
Peritonitis	2.37%	1.91%	1.76%	1.40%	1.97%	1.4%
Unknown causes	1.36%	1.20%	0.67%	0.94%	1.16%	1.1%
*Other diseases	0.03%	0.25%	0.02%	0.27%	1.70%	2.3%
Stress	1.62%	2.91%	0.09%	0.19%	0%	1%
Wild animals	0.27%	0%	0%	0%	0%	0.05%
Mechanical injury	0.08%	0.11%	0.34%	2.21%	3.16%	1.2%
Nutritional deficiency	2.08%	5.38%	9.88%	4.74%	7.10%	5.8%
Coccidiosis	0%	1.13%	0%	0%	0.55%	3.34%
Newcastle disease	0%	0.40%	0.06%	0%	3.96%	4.42%
Total yearly mortality	20.68	64.86	23.36	31.33	22.42	33%

*Other diseases = Those diseases that occurred rarely and were grouped together for example respiratory diseases, *E. coli* infection, anal prolapse, ascitis, osteomalacia, cystic ovary.

From this table it is clear that salmonellosis caused the highest mortalities during this period compared to the other causes of mortalities. Nutritional deficiency is the second cause of mortality to salmonellosis then follows peritonitis, mechanical injury, unknown causes, Newcastle disease, fowl cholera and stress. The other causes of mortalities like other diseases, coccidiosis, wild animals had a very low impact on the mortality rates.

4.2. Cross-sectional

4.2.1. Questionnaire analysis

The layout of the rest of questionnaire is given in Appendix 2. There were thirty questions in all per farm subjected for scoring. Tables 7, 8 and 9 show the results of the questions per farm and grouped according to ownership.

Table 7: Questionnaire results and observations on the status of biosecurity measures in some poultry farms in Debre Zeit.

variables	Farms							
	Large state		Large private			Small private		
	A	B	D	E	H	C	F	G
Foot & vehicle baths at farm entrance (yes=0, no=1)	1	1	1	0	1	1	1	1
Access of wild birds into houses (yes=1, no= 0)	1	1	0	0	1	1	1	1
Free movement of materials in farm (yes=1, no=0)	1	1	0	0	1	0	0	0
Frequent visitors in farm (yes=1, no=0)	1	1	0	0	1	0	0	0
Good hygiene barriers in farm (yes=0, no=1)	1	1	0	0	1	0	0	0
Good sanitation condition in farm (yes=0, o=1)	1	1	0	0	1	0	0	0
Rotation of workers between houses (yes=1, no=0)	1	1	0	0	1	0	0	0
Any species other than poultry on farm (yes=1, no=0)	1	0	0	0	0	1	1	0
Waste deposit in pit (yes=0, no=1)	0	0	1	1	1	1	1	0
Control of flies & vermin (yes=0, no=1)	1	1	1	1	1	1	1	1
Total	9	8	3	2	9	5	5	3

Biosecurity measures can be described as those variables that are likely to spread *Salmonella* infection from one place to the other if they are not kept in good condition and under control. Comparatively it is clearly shown that the large farms especially state owned farms and farm (H) are the least when it comes to biosecurity measures. These farms have the highest scoring. Farm A and H are for layers while farm B is for broilers. No control of flies and vermin is done by any farm. The breeder farms (farm D, E and G) are in a better position when it comes to biosecurity measures followed by farm C and F.

Table 8: Possible sources and predisposing factors to *Salmonella* infection (Hygiene and health status)

	Farms								
variables	Large state		Large private			Small private			
	A	B	D	E	H	C	F	G	
1) Any poultry at 1km from farm (yes=1, no=0)	0	0	1	1	0	1	0	0	
1) Farm surrounded by village (yes=1, no=0)	1	1	1	1	0	1	1	1	
2) Disease problem (yes=1, no=0)	1	1	0	0	1	0	1	0	
1) Cleaning & disinfection done properly (yes=0, no =1)	1	1	0	0	1	0	0	0	
2) Any nutritional deficiency (yes=1, no=0)	1	1	0	0	1	0	0	0	
2) Are birds in good condition (yes=0, no =1)	1	1	0	0	1	0	0	0	
1) Are houses surrounded by clean environment (yes=0, no=1)	1	1	0	1	0	1	1	1	
1) Deposit of waste materials >300m from houses (yes=0,no=1)	1	1	0	0	1	0	0	1	
2) Any salmonellosis prior/ during 96-97 (yes=1, no=0)	0	0	0	0	1	1	1	0	
2) Any mortality reported (yes=1, no=0)	1	1	0	0	1	0	1	0	
Total	8	8	2	3	7	3	5	3	

1) = possible sources
2) = predisposing factors

Large state owned farms seem to have a lower health status than the rest of the farms (Table 8). These poultry farms were crowded and surrounded by villages except farm H. The large farms either being private or state owned had disease problems. Cleaning and disinfection were not properly done on the large farms due to lack of drugs in many occasions. There was a remarkable nutrient deficiency on large farms with retarded growth among birds that were of the same age. Furthermore the birds delayed to reach maturity age. The deposit of waste materials was too close to the rearing units in the case of large farms.

Availability of vaccines and drugs was the problem especially in state farms. Other large private farms manage to import the drugs well in time. Only Newcastle Disease was known and most farms vaccinated against it.

Table 9: Management factors anticipating to contribute to salmonellosis in the studied poultry farm in Debre Zeit

	Farms								
Variables	Large state		Large private			Small private		
	A	B	D	E	H	C	F	G
Recording system (good=0, poor=1)	0	1	1	1	1	1	1	1
Are waste used as manure (yes=1, no=0)	1	1	0	0	1	0	0	0
Are waste used as food additives (yes=1, no=0)	1	1	0	0	0	0	0	0
Clean protective clothing used (yes=0, no=1)	1	1	0	0	1	0	0	0
Are birds same age in house (yes=0, no=1)	1	0	0	0	1	0	0	0
Are birds same breed in house (yes=0, no=1)	1	0	0	0	0	0	0	0
Is sexing of birds done (yes=0, no=1)	1	0	0	0	0	0	0	0
Is litter changed all in all out (yes=0, no=1)	1	1	0	0	0	0	0	0
Is cost & benefit analysis of farm done (yes=0, no=1)	1	1	0	0	1	1	1	1
Market available for farm products (yes=0, no=1)	1	0	0	0	0	0	0	0
Total	9	6	1	1	5	2	2	2

Management plays an important role in production. Poor management induces a lot of problems. Farm A, B and H have poor management as indicated by the score of 5 or above (Table 9). Records were not properly kept by most farms. The large farms used waste material as manure and sometimes as food additives for livestock. Farm A, B and H used protective clothing but these clothes were not changed for a longer time while other farms changed clothing regularly. Farm A and H reared chickens of different ages in one house and furthermore farm A mixed breeds. Sexing of birds was not performed in farm A. To secure a high rate of egg production it is essential to ensure that birds are kept in clean environment to prevent microorganisms to contaminate the surrounding. Farm A and H are for layer production and the conditions in these farms are unlikely to be favorable. Farm A has a hatchery with a capacity of about 1.000.000 per annum but not operating during the period of this study due to recent low hatchability and the high rate of mortality in this hatchery.

To be able to arrange the score points as presented in the Tables 10-13, the class interval width (w) was calculated according to Daniel (1991) using this formula: $w = R/k$

R = (range) which is from 0- 30

k = (number of class interval) is 5. Then $w = 30/5 = 6$

Table 10: Grading and class interval for individual farm

Individual score points for all farms	Grading for the class intervals
0- 6	very good
7-12	good
13- 18	satisfactory
19- 24	bad
25-30	very bad

There are 5 class intervals (from very good to very bad). The denominator is 30 because there are 30 questions per farm. The first range is then from 0-6 and then 6 is added up from one class to the other.

Table 11: Points obtained by individual farm

Farm	Total score points	Grade
A	26	very bad
B	20	bad
C	10	good
D	6	very good
E	6	very good
F	12	good
G	8	good
H	21	bad

All points were added up for each farm. These points were then graded accordingly. As indicated earlier the more points a farm obtained was an indicator that the farm was more at risk. Farm A obtained 26 points in all and was graded as very bad. Farm D and E were graded very good. Farm C, F and G were graded good while farm B and H were considered bad.

For group scoring, two formulas were used because the ranges differ. For large state farms the range is from 0- 60 for farms A and B, whereas in large private farms (D, E, H) and small private farms (C, F, G) the range is from 0- 90 since there are 30 questions per farm and there are three farms in each group so adding all questions it comes up to 90. Table 12 shows the summary of the scoring for both groups

Table 12: Large state farms and large and small private farms grading of class intervals

Large state farm score (points)	Large and small private farms score points	Grading for the class intervals
0- 12	0- 18	very good
13- 25	19- 37	good
26- 38	38- 55	satisfactory
39- 51	56-73	bad
52- 64	74- 92	very bad

The same formula for calculating the class interval width (w) was used but in this case the denominator is 60 for the large state farms because there are 60 variables for these two farms together. The number of class intervals remain the same as above (w= 60/5= 12). The first grade started by 0-12. Then 12 is added in every grading up to the last. The gradates for the large and small private farms are calculated based on 90 variables under the same procedure as for the large state farms.

Table 13: Points obtained by the large state farms and large and small private farms

Farms	Total score points	Grade
Large state farms	46	bad
Large private farms	33	good
Small private farms	30	good

The state owned farms (A and B) obtained 46 points. These points fall in between 39-51 which is graded as bad. Large private farms scored 33 points in all. These 33 points fall in between 19-37 which is graded as good. There is no difference with scores obtained by the small private farms which is 30 and graded as good also. This indicates that the farms under state management have very poor management compared to the rest of the farms. It should not be neglected, that there is also one private farm (H) with a bad situation.

Other measures like monitoring of microbiological quality of water, bacteriological or serological monitoring of chickens were not practiced by all farms. Farm A, B and H used automatic feeders and drinkers while the rest operated manually. The supply of drugs was a big problem especially in state farms where there was a high demand since the number of birds was also large and management was very poor. Most drugs and vaccines are imported and as a result they are not present at all times. But the large private farms (D and E) were well equipped with drugs and vitamins for the birds. Only Newcastle Disease was known by all farms and vaccines have been used except farm C and F since they were new farms.

Layers are kept in cages but there were 4 birds per cage in farm A and 5 birds per cage in farm H. Farm E had hatchery which was very protected. No access of wild birds or strangers in the hatchery building was possible and the sanitation conditions were satisfactory. The hatchery is quite small and easy to handle under our normal conditions. Unlike the hatchery that was in farm A even though it was not operating at the time of the study but it looked difficult to maintain it because it is very big and requires a lot of expenditure of drugs (cleaning and disinfection), it consumed a lot of power and needed very skilled personnel for monitoring. The breeds that were present in farm A were Isa white and brown, in farm B and C was leghorn, farm D had leghorn and lohman, E and F leghorn, G Isa brown and H lohman and Isa brown. For breeding purpose the chicks are imported as day olds. Feed is prepared locally and the ingredients depend on what is available in that season.

4.2.2. Blood sample findings

The objective of the blood sample analysis was to assess the prevalence of S. Gallinarum and Pullorum infection in poultry production. Out of a total of number of twenty flocks included in the cross-sectional study, seventeen flocks were tested for fowl typhoid. Table 14 provides the results.

Table 14: The prevalence of S. Gallinarum-Pullorum using blood agglutination test in poultry farms in Debre Zeit

Farm/ Flock	Total sample size	No blood agglutination positive	Results in percentage
A/1	30	0	0%
A/2	30	0	0%
A/3	30	0	0%
A/4	30	0	0%
A/5	30	0	0%
A/6	30	0	0%
A/7	30	0	0%
A/8	30	0	0%
B/1	30	1	3,3%
B/2	30	0	0%
C/1	30	0	0%
D/1	30	1	3,3%
E/1	30	3	10%
G/1	30	0	0%
H/1	30	0	0%
H/2	30	0	0%
H/3	30	0	0%
Total	510	5	0,98%

Out of 20 flocks selected for the cross-sectional study, there were a total of 17 flocks selected for blood agglutination test. The other three flocks were not tested because they were below two weeks old and therefore not yet immune competent. If one sample is positive the flock is declared positive. A sample size of 30 animals was examined from each flock in order to acquire 95% confidence of including at least one positive sample if the disease is present at 10% level (Cannon and Roe, 1982). Of all 17 flocks there was an overall detection rate of 0.98% with farms/flock B/1, D/1 and E/1 being positive; others were negative. The highest was found in E/1 where three samples out of 30 were positive.

4.2.3. Cultural results

Furthermore a culturing procedure was applied to all the twenty flocks to detect the presence of *Salmonella* species. All samples were pre-enriched in BPW, then enriched in TGB and RV broths and then plated in BPLS and XLD media whereby a very good growth was obtained from these media especially on BPLS.

Out of a total of 315 samples processed, 108 showed suspected colonies which is equal to 44.3%. Suspected colonies were taken for further testing using lysine decarboxylase broth, TSI and Simmon Citrate Agar. For TSI reaction, H_2S, gas, glucose fermentation and alkaline production were observed. The positive samples were both positive to Simmon citrate agar and lysine decarboxylase broth. Afterwards all suspected colonies were tested with Polyvalent sera I and II. From 108 isolates 61 showed a positive reaction to Polyvalent sera I or II which is 56.5%. The isolates were subcultured and tested again using Polyvalent sera I or II. Only 20 isolates were suspected *Salmonella* positive.

The 20 suspected *Salmonella* positive isolates were sent to BgVV for confirmation. From 20 suspected isolates 10 isolates were confirmed positive to *Salmonella*. All the confirmed strains belonged to *S. enterica* subsp. *enterica*. Out of the 10 confirmed isolates 6 of them were under Group D, 2 under Group E and the last 2 were classified as rough stains of *Salmonella*. Table 15 provides an overview of the types of samples taken and the results of the bacteriological examination.

Table 15: Frequency of isolation of *Salmonella* from different samples in poultry in Debre Zeit

Type of sample	No of samples collected (pooled)	Total No of samples collected	Farm codes	Farm and Flock no.	No of suspected +ve	No of confirmed +ve
Foot swab	5	5	A, H	A/1,A/2,A/3,A/6;H/1	0	0
Dust inside pen	20	20	A, H	A/1 to A/8 B/1,B/2,C/1,D/1 to D/3,E/1,F/1,G/1, H/1 to H/3	4	3
Floor sweepings	5	5	A, H	A/1,A/2,A/3,A/6,H/1	0	0
Faecal	300 (50)	50	A, H	A/1,A/2,A/3,A/6,H/1	0	0
Litter	840 (140)	140	A; B; C; D; E; F; G; H	A/4,A/5, A/7, A/8 B/1,B/2,C/1 D/1 to D/3, E/1,F/1,G/1, H/2, H/3	4	3
Liver	140 (14)	14	A, B, D, F, H	A/1 to A/8, B/1,B/2 D/1,D/2, F/1, H/1	3	2
Caecal	140 (14)	14	A, B, D, F, H	A/1 to A/8, B/1,B/2 D/1,D/2, F/1, H/1	1	1
Feed	20	20	A, B, C, D, E, F, G, H	A/1 to A/8,B/1,B/2,C/1,D/1 to D/1,E/1,F/1,G/1, H/1 to H/3	0	0
Water	8	8	A, B, C, D, E, F, G, H	A, B, C, D, E, F, G, H	0	0
Egg-sorting	7	7	A, H, G, E	A/1,A/2,A/3,A/6, A/7, H/1; G/1,E/1	3	0
Drag swab	200 (20)	20	A, B, C, D, E, F, G, H	A/1 to A/8,B/1,B/2,C/1,D/1 to D/3, E/1,F/1,G/1, H/1 to H/3	2	0
Surroundings	8	8	A, B, C, D, E, F, G, H	A, B, C, D, E, F, G, H	2	0
Nest-box	3	3	A, E, G	A/7, E/1, G/1	1	1
Total	315	315			20	10

Table 16 indicates the percentage of positive findings for the different samples taken.

Table 16: Percentage of positive findings of *Salmonella* isolates in poultry from Debre Zeit based on final confirmation

Samples	Number and % of positive findings		
	Total number of samples (pooled)	Number of positive findings	in %
Dust inside pen	20	3	15.0
Litter	1150 (115)	3	2,6
Liver	140 (14)	2	14,3
Caecal	140 (14)	1	7,1
Nest-box	3	1	33,3
Total	**166**	**10**	**6,0**

The most frequently isolated *Salmonella* serovar from these positive samples was S. Enteritidis. *Salmonella* Reference Laboratory at BgVV in Berlin confirmed 60% of the positive strains as being S. Enteritidis. Table 17 shows the breakdown of positive samples.

Table 17: *Salmonella* serovars detected from poultry farms in Debre Zeit

Farm code	Type of production	Age in days	Type of sample	No. of samples (pooled)	No. of samples positive	Sero types
A/7	layer breeder	485	dust inside pen	1	1	SE
B/1	broiler	69	caecal	1 (10)	1	SE
B/1	broiler	69	liver	1 (10)	1	SE
B/2	broiler	49	litter	6 (60)	2	SE
D/1	broiler breeder	140	dust inside pen	1	1	SA
D/2	broiler breeder	6	litter	6 (60)	2	(I)
E/1	broiler breeder	308	nest-box	1	1	SE
F/1	layer	6	litter	6 (60)	3	SE
F/1	layer	6	liver	1 (10)	1	(I)
H/1	layer	522	dust inside pen	1	1	SU

SE = S. Enteritidis
SA = S. Anatum
SU = S. Uganda
(I) = Rough strain

S. Enteritidis was isolated from layer breeder, broilers, broiler breeder, and layer chicks and also from environmental and tissue samples. S. Anatum was isolated from the environmental samples of the broiler breeder, while S. Uganda was detected from layers from the dust inside the house. The rough strains were only identified from the litter samples. Not all the farms were infected by *Salmonella*. Table 18 indicates the farms infected by *Salmonella*.

Table 18: Number of farms/ flocks infected by some *Salmonella* serotypes

Farm	Total flocks investigated	flock positive for *Salmonella*	% infected flock	Type of *Salmonella* isolated
A	8	1	12.5%	S. Enteritidis
B	2	2	100%	S. Enteritidis
C	1	0	0%	No *Salmonella*
D	3	2	66.%	S. Anatum and some rough strains
E	1	1	100%	S. Enteritidis
F	1	1	100%	S. Enteritidis and rough strains
G	1	0	0%	No *Salmonella*
H	3	1	33.%	S. Uganda

From the six farms infected with *Salmonella*, four of them were found positive for S. Enteritidis. Two farms showed rough strains of *Salmonella*, one farm was found positive for S. Uganda and the other to S. Anatum.

The results from the questionnaire survey and bacteriological examination were combined to find out the status of those farms that reacted positive to some *Salmonella* species in Table 19.

Table 19: Comparison of questionnaire and bacteriological results between the farms in the cross-sectional study

Farm scoring category	Farms	Results of the bacteriological examination (positive/ investigated)	% positive farms
Very good and good	C, D, E, F, G	3/5	60%
Very bad and bad	A, B, H	3/3	100%

Out of the 5 farms graded very good and good, there were two farms which were negative to *Salmonella* infection. For those farms that scored from very bad and bad, all of them were positive for *Salmonella*. Table 20 shows the most frequently isolated serovar as being S. Enteritidis.

Table 20: Most frequent *Salmonella* serotypes isolated from poultry farms in Debre Zeit

Salmonella Serotype	No. of positive flocks
S. Enteritidis	4
S. Anatum	1
S. Uganda	1
Rough strains	2

4.3. Longitudinal study analysis

One broiler breeder flock (farm E) was selected for the longitudinal study and followed starting at the hatchery. This farm was one of the positive reactors from fowl typhoid test and also there was S. Enteritidis isolated from the nest boxes sample. Eggs from this farm were followed to the hatchery to find out if *Salmonellae* could be transmitted to the chicks born from this flock. Out of 12 samples taken from the hatchery no *Salmonella* were isolated from the hatchery as in newly hatched chicks. Day old chicks born from this hatchery (farm E1.1

and E1.2) were followed until ready for slaughter. It is important to indicate that day old chicks for farm E1.1 and E1.2 originated from farm E/1 and these two farms were not included in the cross-sectional study. No *Salmonella* were isolated during the rearing period from these two farms. *Salmonella* was only isolated at the end of the rearing period from farm E1.2. No *Salmonellae* were isolated from farm E1.1. There were 131 samples processed from both farms and there were 4 suspected *Salmonella* positive isolates of which one was from farm E1.1 and the rest were from farm E1.2. The 3 samples from farm E1.2 were confirmed positive to *Salmonella* while no *Salmonella* was confirmed from farm E1.1 even though there was suspected *Salmonella* in litter sample from farm E1.1 at the age of 35 days old.

The cases of deaths that were recorded were very few during rearing especially at the early days of chicks. Mostly chicks died due to ascitis as likely result of poor ventilation. Other chicks drowned in the drinkers because of improper placement of the drinkers. or most probably because of very few number of drinkers in a house.

Salmonella species were detected in farm E1.2 at the last stage of production. At the age of 35 days both farms transferred their chickens to new houses within the farm because the other houses were smaller. The reason for infection could be that the new house in which the birds were transferred into was infected and the surrounding was also most likely favourable since there were other livestock reared like local birds, cows, sheep or it could possibly be because of the presence of rats. Table 21 indicates number of samples positive to *Salmonella* at the end of rearing period.

Table 21: Results of the examination of *Salmonella* during rearing period in farm E1.1 and E1.2

		Farm					
		E1.1			E1.2		
samples	No. samples (pooled)	21 d	35 d	49 d	21 d	35 d	49 d
Drag swab	1 (10)	-	-	-	-	-	+
Water	1 liter	-	-	-	-	-	-
Feed	25g	-	-	-	-	-	-
Surroundings	1 (10)	-	-	-	-	-	+
Liver	1 (10)	-	n	n	-	-	n
Litter	6 (25g)	-	-	-	-	-	+

21 d = bacteriological examination of samples at 21 days old
35 d = bacteriological examination of samples at 35 days old
49 d = bacteriological examination of samples at 49 days old
n = no bacteriological examination because no tissue sampled since there were no mortalities
+ = positive findings
- = negative findings
Table 22 shows the type of serovars isolated from farm E1.2.

Table 22: *Salmonella* serotypes detected from the broiler farm (Farm E1.2)

Farm/ flock	Sample collected	Type of *Salmonella*
E1.2	drag swab	S. Enteritidis
E1.2	surroundings	S. Enteritidis
E1.2	litter	S. Uganda

S. Enteritidis was the most frequently isolated serovar followed by S. Uganda in one sample. For the two studies (cross-sectional and longitudinal) S. Enteritidis has been shown to be the most frequent serotype. Another aspect is that S. Uganda has been isolated from the cross-sectional (H/1) and from longitudinal study (E1.2). These flocks are completely different. Flock H/1 consists of layers and flock E1.2 of broilers. These farms are about 5 km away from each other. There is no relationship between the two farms S. Uganda being isolated from. They may share the same source of water but no *Salmonella* was isolated from water. S. Anatum was only isolated in broiler breeder flocks while S. Enteritidis was isolated in layers and in broilers.

4.4. Antimicrobial test results

For all strains isolated from cross-sectional and longitudinal studies, antimicrobial test was done with the seventeen antibiotics. The disk diffusion method was used. Table 23 indicates the antibiotics to which the corresponding serovars were found resistant.

Table 23: Results of antibiotic sensitivity test on *Salmonella* isolated from poultry in Debre Zeit

Flock identity	Sample	Serotype isolated	Drug resistance
A/7	dust inside pen	S. Enteritidis	TC
B/1	ceacal	S. Enteritidis	TC
B/1	liver	S. Enteritidis	TC
B/2	litter	S. Enteritidis	TC
D/1	dust inside pen	S. Anatum	SXT, TC, S W
D/2	litter	Rough strain	Na
E/1	nest-box	S. Enteritidis	sensitive to all drugs tested
F/1	litter	S. Enteritidis	Na
F/1	liver	Rough strain	sensitive to all drugs tested
H/1	dust inside pen	S. Uganda	sensitive to all drugs tested
E1.2	drag swab inside pen	S. Enteritidis	TC
E1.2	surroundings	S. Enteritidis	TC
E1.2	litter	S. Uganda	sensitive to all drugs tested

TC = Tetracycline
SXT = Sulphamethoxazol/Trimethoprim
S = Streptomycin
W = Trimethoprim
Na = Nalixidin acid

There were 8 strains of S. Enteritidis, 1 of S. Anatum, 2 of S. Uganda and 2 rough strains. About 30% of these were sensitive to all drugs (2 strains of S. Uganda (100%), 1 S. Enteritidis (12.5%) and 1 rough strain (50%)). Six strains of S. Enteritidis (75%) were resistant to tetracycline while 1 (12.5%) was resistant to Nalidixin acid. Another rough strain (12.5%) was resistant to Nalidixin acid. S. Anatum strain showed resistance to tetracycline, sulphamethoxazol/trimethoprim and trimethoprim.

5. DISCUSSION

Salmonellosis is considered to be economically important for animals and humans worldwide. Poultry has been reported to be the major reservoir. S. Enteritidis and S. Typhimurium are of the Paratyphoid organisms which cause severe infections in man. S. Enteritidis has been the most frequently isolated serovar followed by S. Typhimurium. Control measures for S. Enteritidis should start at farm level in order to produce *Salmonella* free flock up to the processing and finally to the consumer so that we can reduce infection in man.

Early detection of *Salmonella* is essential. ISO (1993) has established standardized cultural methods in the detection of *Salmonella* using different types of media which are also recommended by WHO (1994). To increase the specificity to 100% by cultural methods the isolates should be sent to a recognized Reference Diagnostic Laboratory for confirmation. Serological methods like ELISA are more sensitive but the specificity may be reduced. The other serological methods WBT and SAT with a stained antigen have been used to detect flocks infected with Group D organisms which might be S. Gallinarum. These tests are very reliable for the detection of infection with S. Gallinarum, but they are of no value looking on S. Enteritidis. In Europe these tests are used on a regular basis to control S. Gallinarum. For other serovars culturing methods are considered being the gold standard methods.

In many occasions *Salmonella* occurrence has been examined using monofactorial approaches i.e. looking only on eggs, feed, housing, water procedures etc. Very limited literature exists on field data and particularly taking into account several risk factors at the same time (Thrusfield, 1986). *Salmonella* infection is a multifactorial syndrome involving various ecological conditions and routes (Noordhuizen *et al.*, 1997). The usefulness of this multivariate approach for the epidemiology of salmonellosis has been advocated by Henken *et al.* (1991).

The present study was conducted to detect *Salmonella* serotypes existing in the study area and to determine the risk factors which might be assumed to contribute to the infection with *Salmonella*. Historical data were analyzed to get information on mortality rates, and productivity over a long time period of 5 years. Time series analysis was done additionally. Thus questionnaires were conducted to get more information about farm management, biosecurity measures, and health status of the chickens in order to reach at scoring of the farms studied.

Retrospective data

In our study analysis of the historical data of farm A clearly demonstrated that salmonellosis is the major disease problem which causes considerable economic losses. There seems to be a pattern of disease outbreaks. In 1992-93 there were *Salmonella* outbreaks almost throughout the year. At the same time mortalities due to fowl cholera, peritonitis, stress and nutritional deficiency occurred. This may point to a relation of *Salmonella* infection with other diseases. Sharma *et al.* (1988) isolated *Salmonella* in chickens which had reproductive disorders including peritonitis. These results support our findings because poor nutrition can lead to reproductive disturbances and consequently to more disease outbreaks.

In 1993-94 and 1995-96 there was a remarkable *Salmonella* outbreak in the month of October. During this month it is dry and hot. In 1994-95 and 1996-97, there was a remarkable *Salmonella* infection outbreak at the onset of the rainy season. This is in agreement with Raó

(1973) who stated that the incidence of *Salmonella* is much higher in summer. However, Rusul *et al.* (1996) found that the incidence of *Salmonella* infection in Malaysia is high at the onset of the rainy season. This study is in agreement with this finding also because the outbreak of salmonellosis started in May which is an extremely hot month and June which is the beginning of rainy season in Ethiopia. Peritonitis, stress, nutrient deficiency and coccidiosis were also observed. This supports the work done by Schobries *et al.* (1989) of isolating *Salmonella* in a flock with a positive diagnosis in peritonitis, nutrient deficiencies, coccidiosis, Newcastle Disease and others. It also explains that higher temperatures allow *Salmonellae* which might be on or in the egg to multiply to the extent that they can be detected.

Elevated temperatures in layer houses during summer increase the frequency magnitude, the duration of external or internal egg contamination with *Salmonella* due to stress for the laying hens. In Ethiopia there are only two major seasons; the wet and the dry season. It is obvious that salmonellosis can occur in any season. There are some years that experienced *Salmonella* infection in all months throughout the year. The significant difference of mortality due to salmonellosis between months observed in this study period shows that control measures should aim at eliminating these outbreaks in some months as a first step.

Gorham *et al.* (1994) inoculated S. Enteritidis phage type 13A on chickens and observed among the postmortem lesions also peritonitis. Bercheiri *et al.* (1992) reported cases of S. Typhimurium with peritonitis among other symptoms. This supports the relationship between salmonellosis outbreaks and other concurrent diseases. Though salmonellosis is not the only disease existing in this farm at least a higher percentage of mortality is assumed to be attributed to salmonellosis. The highest mortality was observed in 1993-94 whereby 55% out of 75% total mortality can be attributed to salmonellosis.

There seems to be a relation between egg production and mortality rates. Comparing the two production cycles, different patterns could be observed. In one cycle there is no real influence of mortality due to salmonellosis or other disease on egg production. In the other cycle a high mortality due to salmonellosis and other diseases was closely connected with a considerable decrease in egg production. This is in agreement with Luthgen, (1979) where S. Gallinarum was reported to have caused a heavy loss in egg production among laying hens in Germany in 1976. These layers were at the end of laying period. Hinz *et al.* (1991) isolated S. Enteritidis among *Salmonella* serovars which were present in layers at the end of laying period.

Questionnaire analysis

The aim of the questionnaire analysis was to quantify the contributory effect of several risk factors for *Salmonella* occurrence in these farms. The study was conducted during a period of 5 months and a total of twenty flocks were included in the cross-sectional study. Based on this information one parent flock and the corresponding hatchery and two rearing flocks were selected for the longitudinal study. Henken *et al.* (1996) incorporated 111 breeder flocks for a period of five years to analyze the contributory risk factors to salmonellosis, applying multivariate analysis using logistic regression models to quantify the risk of disease occurrence.

In the present study further statistical analysis could not be applied due to the small number of flocks (20 flocks) in the cross-sectional study and 2 flocks in the longitudinal study which could be investigated within the time scheduled of five months. This was too short to

determine some risk factors and to include further farms. In the cross-sectional study analysis of the available data gave a clear and quantitative picture on differences in the status of these farms under different management conditions. It became obvious that the state owned farms have very poor management in general when applying scoring procedures. Pellaz (1996) used scoring to be able to classify the farms based on hygiene standards. As biosecurity measures are not kept well in our farms studied, the farms run a high risk of acquiring S. Enteritidis infection as it is more often associated with layers (Reiber and Conner, 1994; Corrier *et al*, 1992). Also one aspect which has to be considered is that this is the first study of this kind conducted in this area and therefore a lot of missing data were encountered. It has to be clearly stated that this study is not representative for the poultry population in Ethiopia. It was done only in a smaller part of the country.

Blood agglutination test

In the cross-sectional study, blood agglutination test results show three positive flocks. These data clearly confirm that S. Gallinarum is prevalent in some flocks and may be responsible for the *Salmonella* outbreaks with a high mortality rate observed in the historical data analysis. S. Gallinarum could not be isolated from flocks. This does not indicate that the flocks were negative. There might be some problems with the cultivation. It is with the agreement with Lahellec and Colin (1985) demonstrating that negative reaction does not mean the flocks are free of *Salmonella*. This result also depicts that the blood agglutination test is not a reliable method to detect infection caused by other *Salmonella* serotypes. To get an information on this culture techniques are more appropriate. Out of seventeen flocks three flocks became positive in culture as well as in blood agglutination. Flock A/7, B/2 and H/1 were negative to the blood agglutination test but were found positive in the culture. This confirms again that agglutination is not a reliable test (Bergguist *et al.*, 1973).

Cultural results

Salmonella in poultry has been the subject of investigation for many years. Currently, problems are arising with the great number of facultatively pathogenic *Salmonella* species because of the increased hazard to human health. There are several approaches applied to detect *Salmonella* organisms. ISO (1993) has established standardized methods to be followed in the isolation of *Salmonella* which include the resuscitating stressed *Salmonella* before transferring to a selective medium. In the present study BPW was used for pre-enrichment as proposed by Dalsgaard *et al.* (1995).

The need to isolate *Salmonella* from non clinical samples and from the environment has resulted in the development of more sensitive and selective media for isolating *Salmonella* species. Waltmal *et al.* (1994) determined in their survey the extent of variability between 34 states laboratories in the United States providing *Salmonella* diagnostic service to the poultry industry. For environmental samples 79% used TGB- broth, and 38% used selenite broth. According to Bailey and Cox (1996) selenite and TGB-broths are commonly used in the United States while RV-broth is mostly used in Europe. In the presented study TGB and RV-broths were used for enrichment. This is in contrast to ISO (1993) where selenite and RV-broths are recommended. In a study on *Salmonella* prevalence in poultry, Müller *et al.* (1997) reached a higher sensitivity using the media as done in our study. Dalsgaard *et al.* (1995) demonstrated the failure not to use at least two different media affected his final results of isolating *Salmonella*.

For plating out, at least two media are recommended by ISO. Phenol-red lactose-sucrose agar (BPLS) is compulsory plus any other media of choice (ISO, 1993). Many laboratories use BPLS together with a media containing hydrogen sulfide (H_2S) in order to be able to detect those *Salmonellae* positive to H_2S that might be missed by BPLS. Johansson (1996) used XLD which contain H_2S and BPLS for plating. The same were used in the present study. For tissue samples the most used media according to Waltman *et al.* (1994) were MAC agar, BPLS, XLT4 which contains H_2S. In this study MAC agar was used. The suspected colonies are inoculated into TSI agar and lysine decarboxylase broth. These were applied also in this study as well as sending the suspected *Salmonella* positive isolates to the Reference Laboratory for final confirmation.

Serotypes isolated

Generally, the results obtained from this study show that more than 60% of the isolates were S. Enteritidis thus the predominating serovar in the study area. This is in agreement with Rusul *et al.* (1996). No S. Typhimurium was isolated from any sample. Notermans *et al.* (1996) indicated that the most frequently isolated serovar in 60 to 80% of all *Salmonella* isolates is S. Enteritidis, which has now replaced S. Typhimurium in many countries. In the cross-sectional study *Salmonella* was isolated from dust inside pen, litter, nest-box, liver and ceacal of which 60% of the isolates were S. Enteritidis. No *Salmonella* was isolated from faecal samples.

Earlier studies showed that a higher percentage of dust and eggbelt samples were positive to *Salmonella* than faecal samples (Poppe *et al.*, 1991). The reason might be due to the abundant microflora in faecal samples which affects the sensitivity by overgrowing the flora of interest (*Salmonella*) (Noordhuizen *et al.*, 1997). No *Salmonella* was isolated in foot swab, floor sweepings, feed, water, drag swab, egg sorting tables and surroundings. Though a number of samples were negative this does not mean that the flocks are *Salmonella* free because the number of samples taken does not allow to detect a low infection rate. Furthermore some serotypes may persist in the environment without being isolated by this procedure (Lahellec and Colin, 1985).

Different serotypes are prevalent, which shows that there are several sources of infection. Several flocks are infected at all production types. This confirms that humans are exposed to *Salmonella* infection, both via eggs and poultry meat. As host adapted serovars and not host adapted serovars are prevalent, the enormous economic losses, described in the historical data analysis may be caused by both types of *Salmonella*. In general, in the cross-sectional study the risk factors were categorized in biosecurity measures, hygiene and health status and management. These enabled us to classify the farms by scoring procedures. Farms A, B and H were graded as very bad and bad. From all these farms *Salmonella* was isolated. This result shows that in private farms the situation is depending on the persons responsible for it and may be as worse as in state farms. But it became obvious that large farms can be managed as good as small private farms as this is the case with farms D and E.
Farms C, D, E, F and G were under very good and good category. *Salmonella* was isolated in three of these farms which makes 60%. Farms C and G were negative. This supports a point that the farms which were categorized as very bad and bad were in a higher risk than those that were very good and good. According to Swart (1995), S. Enteritidis can be controlled by keeping biosecurity measures in good condition. Our results are in agreement with this statement because these measures are not kept well and S. Enteritidis was the most frequently isolated serovar. Other serovars that were isolated were S. Anatum and S. Uganda.

Furthermore the frequent occurrence of salmonellosis for consecutive years indicated in historical analysis and the economical losses indicated in historical analysis prove this point. Henriksson (1992) demonstrated the prevention of diseases by use of sanitation program, good management, good feeding and housing as the most economical method.

Longitudinal study

S. Enteritidis was detected from the nest boxes of the broiler parent stock (E/1) which was selected for the longitudinal study. This flock was also reacting positively in the blood agglutination test. As other samples taken in this flock were *Salmonella* negative, infection rate may be low. At the hatchery there were no *Salmonellae* isolated even at the early days of live of the chicks born from this flock. Figures on the hatchability (i.e. number of deaths in shell) are not available. But at the time of the study, there were no clinical signs of typhoid in the parent stock and no increased mortality in the day old chicks. Thus, it is not astonishing that S. Gallinarum or S. Enteritidis could not be isolated, as infection may be prevalent at a low rate. The results of this study are described to estimate the recent situation in a flock. Although *Salmonellae* were detected in the parents, the chicks hatched from these flocks were not *Salmonella* positive. Moreover, there were no *Salmonellae* detected from faecal samples of the parent flock, and the hatchery standards were satisfactory. Also the S. Enteritidis strain that was isolated from farm E/1 (nest boxes) was sensitive to all drugs tested.

Testing of eggs before hatched was not carried out since the purpose of the study was to observe the transmission pattern from an infected flock to their progeny. *Salmonella* is thought to penetrate through the shell via faecal contamination of the shell surface (Reiber and Conner, 1994). Bichler *et al.* (1996) indicated that the degree of S. Enteritidis infection in an egg layer flock and the probability that the laying hens will produce contaminated eggs is not clear. Egg collection on this farm E is done regularly resulting in a reduction of the chance of *Salmonella* to penetrate through the egg and contaminate the egg via horizontal route. However, transovarian transmission (vertical transmission) has been found to occur rarely (Bichler *et al.*, 1996; McLlroy, 1996).

Salmonella species were isolated at the end of the rearing period in one of the selected farms (E1/2). The most predominant serovar was again S. Enteritidis. Furthermore, S. Uganda was detected. These findings clearly demonstrate, that there were several sources of infection. As no comparison of S. Enteritidis isolates derived from the parent stock and the broiler flocks (E1.2) using molecular analysis could be performed, it is not clear whether this strain was transmitted at a low level from the parent stock or a new infection had occurred.

Lahellec and Colin (1985) found in their longitudinal study that the incidence of *Salmonella* positive samples occurred between hatching and the first week of the rearing period and between the end of the rearing period and processing. In this study *Salmonella* was found only at the end of the rearing period on the farm. The chickens were transferred at the age of 35 days into a new house. This house was situated on the other part of the farm where other livestock like cattle, dogs, cats as well as sheep were kept too. Mice have shown to be S. Enteritidis carriers.

There were some rats around. Infection might have been transmitted by these. In contrast, on the other farm (E1/1) the chickens were also transferred into a bigger house but the surrounding was clean. No rearing of other livestock took place on that farm. No information is available on rodents on this farm. The results of this study are in agreement with other

findings that *Salmonella* infection may be acquired during the rearing period and highest prevalence is reached at the end of the fattening period (Müller *et al.*, 1997). It confirms the public health hazard due to S. Enteritidis contaminating poultry meat.

Antimicrobial resistance

Among the antibiotics tested, the strains have shown to be most frequently resistant to tetracycline. The occurrence of *Salmonella* resistance to tetracycline in our isolates was not astonishing because this drug is the most commonly used. This is in agreement with Hummel, (1979) on his findings. Lee *et al.* (1993) discovered in their investigation that *Salmonella* strains isolated from healthy broiler chickens were found resistant to tetracycline, streptomycin and trimethoprim/sulfamethoxole before slaughter. This finding is in agreement with our study also.

Some limitations were encountered in the study presented. Identification of farms to be included in the study was one of the biggest constraints encountered as this had been the first study of its kind. As a result the number of farms to be included was limited. During data collection some data were missing which could have been used in the historical analysis to have a picture of *Salmonella* behavior in the past. Mortality diagnosis was based on clinical observations only, no laboratory confirmation has been done. Therefore, no information was available on the *Salmonella* serovars which may have caused the clinical disease.

Furthermore, figures on productivity as an additional variable to calculate the effect of clinical disease were not stored. Historical data could be collected only from one farm. In the others, record keeping was not done on a regular basis. In farm A, mainly mortality statistics were stored. In some years, egg production was registered too. Other data were not available for analysis. Appendix 3 shows the suggested recording formats especially for the private farms. Insufficient laboratory facilities and chemicals also played a very important role in the processing of the samples resulting in a very small amount of samples that were processed at a time. Time constraints as indicated earlier were the main problem in this study to fully achieve the initially intended goals of the study.

6. CONCLUSIONS AND RECOMMENDATIONS

When drawing conclusions it is important to indicate that the population under study was a selected one that does not represent the general poultry population in Ethiopia. In this present study it appeared that, in quantifying the contributory effect of risk factors for the salmonellosis occurrence, a longer period of investigation is required and as well as a representative sample size. This study was successful in demonstrating both, the economical problem and the public health risk in poultry production in Ethiopia.

The isolation of S. Enteritidis, S. Uganda and S. Anatum emphasizes the threat to food poisoning in the area, whereas 18% of positive flocks to S. Gallinarum-Pullorum indicate the economical loss. These serotypes have been isolated in all different types of production, being in layers or broilers. This may result in a high risk of infection either via consumption of eggs or poultry meat. S. Enteritidis has shown to be the most prevalent serotype among the investigated flocks. Therefore a potential public health hazard due to S. Enteritidis is obvious.

The small private farms are in a fair condition compared to the large farms. The fact that two flocks from the cross-sectional study and one flock from the longitudinal study remained free from *Salmonella* infection should encourage farmers to continue their efforts in producing poultry under good hygiene practices.

Several recommendations are suggested as follows:

- Improvements are required in farm management (and husbandry practices) in organization of the poultry industry as a whole, with emphasis on hygiene, good housing, eliminating multiage grouping of birds, proper feeding, water, and less use of drugs, particularly antibiotics.
- Any flock that contains positive reactors to fowl typhoid should be depopulated to decrease the chances of carrier birds from spreading the disease.
- For further investigations, there should be improvement of laboratory techniques for the detection and typing of *Salmonella*.
- More detailed research on major risk factors involved in infection of poultry is required.
- *Salmonella* has many serotypes of which some are highly pathogenic to human beings. Therefore information is required on the incidence of *Salmonella* infection in humans, source of infection and the serotypes involved.
- Further studies are required to characterize the types of strains existing in this area in order to apply appropriate measures of control.
- Improvement on at least the basic record keeping as suggested in Appendix 4 is very essential.

7. REFERENCES

Alamargot, J. (1987): Avian pathology of industrial farms in Ethiopia. IRA Proc. First Natl Livestock Conference, Feb. 1987. Addis Ababa: IRA Ethiopia pp 11-13.

Andrian, N. N., Delaat, A. R. T. (1979): Microbiology of the Allied Health Professionals. 2nd ed. Lea and Fibiger, 203-206.

Anonymous, Directeur Diegesuntheit. (1993): S. Enteritidis. WHO/ CDS/ VPH/, 93 pp 123. Geneva. World Health Organization, pp 123.

Anonymous. (1989): Eradication of *Salmonella* from the hybrid birds. Poult. Int. 3, 1988- 89.

Arbeitskreis für Veterinärmedizinische Infektionsdiagnostik, (AVID) (1996):"Mikrobiologie, Parasitologie und Hygiene" der Deutschen Veterinärmedizinischen Gesellschaft; Loseblattsammlung Methoden der Infektionsdiagnostik III/ 1996.

Arnall, L., Keymer, I. F. (1975): Salmonellosis : Birds Dis. Baillière Tindall, pp 113-120.

Bahatia, T. R. S., Mc Nabb, Wyment G. D., Nayer, G. P. S. (1986): *Salmonella* isolated from litter as indicator of flock infection and carcass contamination. Avian Dis. 24, 833-837.

Bailey, J, S., Cox, N. A. (1996): Detecting specific *Salmonella* strains. World Poult. May, 18-19.

Bailey, J. S. (1993): Control of *Salmonella* and *Campylobacter* in poultry production. A summary of work at Russel Research Center. Poult. Sci. 72, 1169- 1173.

Bailey, J. S., Cox, N. A., Berrang, M. E. (1994): Hatchery-acquired *Salmonellae* in broiler chicks. Poult. Sci. 73, 1153- 1157.

Barrow, P. (1997): Int. *Salmonella* Symposium-Ploufragan '97. Poult. Int. 36, (No. 13) 19-20.

Bean, N. H., Griffin, P. M. (1990): Food-borne disease outbreaks pathogen, vehicles and trends Food Pro. 53, 804-817.

Bercheiri, J. A., De- Carvalho, A. M- ., Fernandez, S. A., Iba, A. M. (1992). Detection of *Salmonella* Typhimurium in a broiler chicken flock. Revista de Microbiol. 24, 212-213.

Bergguist, E., Rosende, S., Bauer, R. (1973): Factors which can disturb the haemagglutination test in the diagnosis of pullorum disease isolation and identification of microorganisms which react nonspecifically with pullorum antigen. Agric. Tec. (Santiago) 33, 204-208.

Biberstein, E. L., Zee, Y. C. (1990): Salmonellosis. In: Smith, A. D., Miller, C. (eds): Veterinary Medicine, 7th edition. London: May and Baker, pp 643-55.

Bichler, L. A., Nagaraja, K. V., Halvorso, D. A. (1996): *S.* Enteritidis in eggs, cloacal swab specimens and internal organs of experimentally infected White Leghorn Chickens. Am. J. Vet. Res. 57 (No. 4), 489- 494.

Boachie, F. (1983): Notes on *Salmonellae* isolated from poultry in Accra, Ghana Bull. Anim Health. Prod. Afr. 33, 39- 42.

Borland, E. D. (1995): *Salmonella* infection in poultry. Vet. Rec. 97, 406-408.

Bouzoubaa, K., Lemainguer, K., Bell, J. W. (1991): Village chickens as a reservoir of *S.* Pullorum and *S.* Gallinarum in Morocco. Prev. Vet. Med. 12, 95- 100.

Bryan, F. L (1988): Risk associated with vehicles of food-borne toxin. J. Food Pro. 51, 498-508.

Buxton, A., Fraser, G. (1977): *Salmonella* In: Anim. Microbiol. In: Immunology, Bacteriology, Mycology Diseases of Fish and Laboratory Methods. Blackwell Sci. Publ. Vol 1 pp 103-114.

Cannon, R. M., Roe, R. T. (1982): Livestock Disease survey: A field manual for veterinarians Australian Government Publ. Canberra, p 16.

Cason, J. A., Cox, N, A., Bailey, J. S. (1993): Ecology and implications of *Salmonella* - contaminated hatching eggs. In: Proc. XIX World's Poult. Congress, Amsterdam, The Netherlands pp 72- 75

Chauhan, H. V. S. (1993): Poultry Diseases, Diagnosis and Treatment. New Delhi, pp 13

Coetzee, L., Swart, J., Etherington. (1993): Presentation at Congress by *S.* Enteritidis Task Group -(SAPA) Konkres. Plumvee- Bull Junie, 233.

Corrier, D. E., Hinton, A. Jr., Hargis, B., Deloach, J. R. (1992): Effect of used litter from floor pens of adult broiler *Salmonella* colonization of broiler chicks. Avian Dis. 36, 897-902.

Dalsgaard, A., Huss, H. H., Kittiku, A. H., Larsen, J. L. (1995): Prevalence of Vibrio Cholera and *Salmonella* in a major shrimp production area in Thailand. Intl. J. Food. Microbiol. 28, 101-113.

Daniel, W. W. (1991): Biostatistics: A foundation for Analysis in Health Sciences 5th edition. New York: John Willy and Sons

Ewing, H. W. (1986): Edwards and Ewing's Identification of *Enterobacteriaceae*. 4th ed. New York: Elsevier Sci. Publ. Co., Inc.

FAO/ WHO. (1992): WHO surveillance program for control of food-borne infections and intoxication in Europe, 6th edition. Report 1990- 92 Berlin, Germany, pp 203-216.

FAO/ World Bank. (1993): Ethiopian Livestock Sector Development Report No: 24/ 93 CP. ETHI 45 Sr,. Ethiopian Sector Development Project. Vol. 1 of 2. Food and WHO/UN.

Finley, B. B., Falkow, S. (1988): *Salomonella* as an intracellular parasite. Mol. Microbiol. 3. 1822-1834.

Galan, J. E., Curtis III. (1989): Cloning and Molecular characterization of genes whose production allow *S.* Typhimurium to tissue culture cells. Proc. Natl. Acad. Sci. USA 86, pp 6383-6387.

Gast, R. K., Beard, C. W. (1993): Research to understand and control *S.* Enteritidis in chickens and eggs. Poult. Sci. 72, 1157- 1163.

Gordon, R. F. (1976): Poult. Dis. London: Baillière Tindall.

Gorham, S. L., Kadavil, K., Vaughan, E., Lambert, H., Abel, J., Pert, B. (1994): Gross and microscopic lesions in young chickens experimentally infected with *Salmonella* Enteritidis. Avian Dis. 38, 816- 821.

Hassan, J. O., Barrow, P. A., Mockett, A. P .A., Macleod, S. (1990): Antibody response to experimental *S.* Typhimurium in chickens measured by ELISA. Vet. Rec. 126, 519-522.

Helmuth, R., Schroeter, A. (1994): Mol. typing methods for *S.* Enteritidis. Int. J. Food Microbiol. 21, 6677.

Henken, A. M., Frankena, J. O., Goelema, Graat, E. A. M., Noordhuizen, J. P. T. M. (1991): Multivariate epidemiological approach to salmonellosis in broiler breeder flocks. Poult. Sc. 71, 838- 843.

Henriksson, R. (1992): Building and management systems for broiler and layers. 9 UNDP/ OPS. Regional Program RAF/ 88/ R51 pp 1-126.

Herenda, D., Chamber, P. G., Etriqui, A., Seneviratina, P. (1994): Manual on meat inspection for developing countries. Animal Production and Health Paper 119. Rome: FAO, pp 328-331

Higgins, R., Malo, R., Rnr-Boberge, E., Gunther, R. (1981): Studies on the dissemination of *Salmonella* in nine broiler chicken flocks. Avian Dis. 26, pp 26- 33.

Himathongkham, S., Gracas, P. M., Riemann, H. (1996): Heat destruction of *Salmonella* in poultry feed, effect of time, temperature and moisture. Avian Dis. 40, 72-77.

Hinz, K. H., Legutko, P., Schroeter, A., Lehmacher. W., Hartung, M. (1996): Prevalence of motile *Salmonellae* in egg- laying hens at the end of the laying period. Zentralbl. Veterinärmed [B] (Germany) 43 (1), 23- 33.

Hirsh, D. C. (1990): *Salmonella*. Blackwell Sci. Publ. Rev. Vet. Microbiol, 2, 110- 111.

Hobbs, B. C., Gilbert, R. J. (1978): Salmonellosis. Blackwell Sci. Publ. Food poisoning and Food Hygiene. 4th ed. pp 41-47.

Hummel, P. H. (1979): Antibiotic resistance among *Salmonellae* isolated from animals in Tanzania. Bull. Anim. Health. Prod. Afr. 27, 113- 121.

Humphrey, T. T., Mead, G. C., (1988): Poultry meat as source of human Salmonellosis in England and Wales. Epidemiol. Infect. 100, 175- 184.

International Livestock Centre for Africa (ILCA). (1988): "An Introduction to ILCA's Debre Zeit Research Station" Addis Ababa, Ethiopia.

International Standard Organization (ISO). (1993): Microbiol.- General guidance on methods for the detection of *Salmonella* (ISO. 6579).

Jansen, J. (1990): On the statistical analysis of ordinal data when extra variation is present Appl. Stat. 39 (1), 75- 84.

Johansson, T. M. -L., Schild, R., Ali-Yrkko., Siitonen, A., Maijala, R. L. (1996): The first S. Enteritidis phage type 1 infection of a commercial layer flock in Finland. Acta Vet. Scand. 37, 471- 480.

Jones, F. T., Axtell, R. C., Rives, D. V., Shideler, R. F., Tarver, J. R. (1991): The survey of *Salmonella* contamination in modern broiler production.Prot. 54, 502- 507.

Julian, M. (1996): What makes S. Enteritidis stick in chicks? World Poult. May, 22- 23.

Käsbohrer, A., Blaha, T. (1997): Field experiences with Monitoring and Reducing *Salmonella* in a Poultry Meat Production Chain. Proc. *Salmonella* and Salmonellosis May 20-22 Zoopôle Ploufraga France pp 626- 630.

Käsbohrer, A., Talaska, T. H., Bögel, K., Stoehr, K., Lehmacher, W. (1993): Epidemiologische Muster von S. Enteritidis und S. Typhimurium beim Menschen In: Proc. Veterinärepidemiologisches Seminar, Hannover Tierärztliche Hochschule, Hannover.

Kelterborn, E. (1992): Kauffmann- White Scheme (1989) Institut für Veterinärmedizin im Bundesgesundheitsamt Berlin und Staatliches Veterinäruntersuchungs- und Tiergesundheitsamt Chemnitz.

Ketchum, P. A. (1988): Typhoid fever, a *Salmonella* Bacteria Microbiol. Concept and Applications Oakland University pp 604- 611.

Lahellec, C., Colin, P. (1985): Relationship between serotypes of *Salmonella* from hatcheries and rearing farms and those processing poultry carcasses. Br. Poult. Sci. 26, 178- 186.

Lahellec, C., Colin, P., Bennejean. G., Paquin, J., Guillerm, A., Debois, L. C. (1986): Influence of resident *Salmonella* on contamination of broiler flocks. Poult. Sci. 65, 2034- 2037.

Le Minor, L. (1984): *Salmonella*. In: Krieg, N. R. and Holt, J. G., (eds): Bergey's Manual of Systematic Bacteriology. Vol. 1. Baltimore, London: Williams & Wilkins.

Le Minor, L. (1988): Typing of *Salmonella* Species. Europ. J. Clin. Microbiol. Infect. 7, 214- 218.

Le Minor, L., Popoff, M. Y. (1987): Designation of *Salmonella enterica* sp. nov., nom., nom. rev., as the type and only species of the genus *Salmonella*. Int. J. Syst. Bacteriol. 37, 465- 468

Lee, L. A., Threatt, V. L., Puhr, N. D., Levine, P., Ferris, K., Tauxe, R, V. (1993): Antimicrobial resistant *Salmonella* species isolated from healthy broiler chickens after slaughter. J. Am. Vet. Med. Assoc. 202, 752- 755.

Lee, M. L., Hung, H. H., Tsang, C. T., Lin, M. Y. (1994): Reduction of the egg transmission of *S.* Pullorum by desinfection Taiwan. J. Vet. Med. Anim. Husband. 64, 23.

Luthgen, W. (1976): *Salmonella* Pullorum infection in a flock of laying hens. Dtsch Tierärztl. Wochenschr. 86 (2), 62- 65.

Mackenzie, M. A., Bins, B. S. (1976): Dissemination of *Salmonella* serotypes from raw feed ingredients to chicken carcasses. Poult. Sci. 55, 957- 960.

Mallinson, E. T., Snoeyenbos, G. H. (1989): Isolation and identification of avian pathogens 3rd ed. Am. Assoc. Avian Pathologists pp 3- 10.

McLlroy, S. G. (1996): How do birds become infected by *Salmonella* serotypes? World Poult. May, 15- 17.

Mead, G. C. (1990): Food poisoning *Salmonella* in the meat industry. Br. Food J. 92, 32- 36.

Microsoft Encarta. (1994): Ethiopian Microsoft Corporation. Funk and Wagnalls Corporation.

Müller, K., Käsbohrer, A., Blaha, T. (1997): Prüfungen der Modifikation des Salmonellennachweises nach ISO 6579 auf ihre Anwendbarkeit für Monitoring-Untersuchungen in Geflügelfleischproduktionsketten. Fleischwirtschaft 77 (6), 563-567.

Ndzinge, I. T., Mary, L Hall, M., Mares, R. G. (1982). Isolation of *Salmonella* from meat and meat products in Botswana.Bull. Anim. Health. Prod. Afr. 27, 166-167.

Noordhuizen, J. P. T. M., Frankena, K., van der Hooft, C. M., Graaf, E. A. M. (1997): Veterinary Epidemiology and Foodborne Diseases. Appl. of Quantitative Methods in Veterinary Epidemiology. Wageningen: Wageningen Pers., pp 273- 292.

Notermans, S., Teunia, P., Borgdoff, M., Van de Giessen, A., Amen, J. H. A. (1996): The cost- benefit analysis of S. Enteritidis eradication program. World Poult. May, 10- 14.

Novick, R. P., Clowes, R. C. Cohen, S. N., Curtis III R., Datta, N., Falkow, S. (1976): Uniform nomenclature for bacteria plasmids, a proposal. Bact Ref. 40, 168- 89.

Nurmi, E., Nuotio, L. (1994): Safety requirements for commercial competitive exclusion products. Proc. European Poult.Conference Glasgow 7- 12th August, 1994.

Old, D. C. (1989): *Salmonella* In: Wilson, G. S. and Miles, A. Principles of Bacteriology Virology & Immunology 6th ed. Vol. 2 Tropley & Wilson, pp 915- 955.

Olsen, J. E., Brown, D. J., Skov, M. V., Christensen, J. P. (1993): Bacterial typing methods suitable for epidemiological analysis. Applications in investigations of salmonellosis among livestock. Q. J. Vet. Sci. 15, 125- 135.

Osiyemi, T. I. O. (1975): Salmonellosis in Nigeria Federal Department of Vet. Research Vom. Nigeria, pp 261- 264.

Pellaz, V. (1996): Der Einfluß eines Darmflorapräparates und von Risikofaktoren auf das Vorkommen von Salmonellen in Geflügelmastbeständen. Doctoral Thesis Tierärztliche Hochschule Hannover.

Petrak, M. I. (1982): *Salmonella* as intestinal pathogen disease of cage and aviary birds 2nd ed. Philadelphia: Lea. & Febriger, pp 504- 508.

Popoff, M. Y., Bockemühl, J., Hickman- Brenner, F. W. (1996): Supplement 1995 (no. 39) to the Kauffmann- White Scheme. Res. Microbiol. 147, 765-769.

Poppe, C., Elliot, L. A., Duncan, C. L. (1996): Evaluation of immunomagnetic separation in combination with modified semi-solid Rappaport Vassiliadis medium and Rampach agar for the isolation of *Salmonella*. J. Microbiol. method 25, 237- 244.

Poppe, C., Irwin, R J., Forsberg, C. M., Clarke, R. C., Oggel, J. (1991): The prevalence of *Salmonella* Enteritidis and other *Salmonella* spp. among Canadian registered commercial layers flocks. Epidemiol. Infect. 106, 259- 270.

Qin, Z. R., Fukata, T. Baba, E., Arakawa, A. (1995): Effect of *Eimeria tenella* infection on *Salmonella* Enteritidis infection in chickens. Poult. Sci. 74, 1- 7.

Quinn, P. J., Carter, M. E., Markey, B., Cater, G. C. (1995): Salmonellosis. In : Clinical Veterinary Micobiol. Walfe, pp 226- 234.

Rao, V. D. P. (1973): *Salmonella* serotypes in poultry and their public health significance Indian J. Anim Health. 17, 43-46.

Read, S. C., Irwin, R. J., Poppe, C., Harris. (1994): A comparison of two methods from isolation of *Salmonella* from poultry litter samples. Poult. Sci. 73, 1617- 1622.

Reeves, M. W., Evins, G. M., Plikasaytis, B. D., Farmer, III. J. J. (1989): Clonal nature of *Salmonella* Typhi and its genetic relatedness to other *Salmonellae* as shown by multilocus enzyme electrophoresis, and proposal of *Salmonella bongori* comb. nov. J. Clin. Micobiol. 27, 313- 320.

Reiber, M. A., Conner, D. E. (1994): Effect of mating activity on the ability of S. Enteritidis to persist in the ovary of chickens. Avian Dis. 39,. 323- 327.

Rengel, A., Mendoza, S. (1984): Isolation of *Salmonella* from raw chickens in Venezuela. J. Food. Prot. 43: 3, 213-216.

Ricci, N., Manuppella, A., Laurelli, T., Durante, M., Trillo, P., Simeone, R. (1990): Case of food poisoning from locally produced ice cream, attributed to a rare *Salmonella* serotype, *Salmonella* Uganda. Igiene Moderna 2, 240- 244.

Rusul, G., Khair, J., Radu, S., Cheah, C. T., Yassin, R. Md. (1996): Prevalence of *Salmonella* in broiler retailed outlets, processing plants and farms in Malaysia. Int. J. Food Microbiol.33, 183- 194

Schiemann, D. A., Montgomery, A. L. (1992): Immune response in chickens against S. Typhimurium monitored with egg antibodies. Vet. Microbiol. 27, 295- 308.

Schobries, H., Schulze, L., Meyer, A. (1989): Disease status of fowls in smallholdings. Monatshefte für Veterinärmedizin. 44: 14, 506- 509.

Schwabe, C. W. (1984): Vet. Med. and human Health 3rd. ed. Williams and Wilkins

Seifert, H. S. H. (1996): Salmonellosis. In Seifert, H. S. H. (ed). Trop. Anim. Health. Doratrecht: Klumer Academic Publ. pp 368- 371.

Sewell, M. M. H., Brocklesby, D. W. (1990): Book on Animal Dis. in the Trop. 4th ed. London: Baillière Tindall pp 92- 96.

Sharma, J. K., Joshi, D, V., Baxi, K, K. (1988): Studies on drug sensitivity pattern of bacteria related with reproductive disorders of poultry. Indian J. Poult. Sc. 15, 116- 118.

Shivaprasad, H., Morales, S., Lucio, B., Baker, R. C. (1990): Pathogenesis of S. Enteritidis infection in layer chickens, studies on egg clinical signs, fecal shedding and serologic responses. Avian Dis. 34, 548- 557.

Silliker, J. H. (1980): Status of *Salmonella* ten years later. J. Food Prot. 43, 307- 313.

Swamy, S. C., Barnhart, H. M., Dreesen, D. W. (1996): Appl. Env. Microbiol. pp 3768- 3771.

Swart, J. C. (1995): S. Enteritidis in South Africa- An update. Plumvee Bull Oktober, 454.

Teshome, M., Mebratu, G/Y. (1988): A study of the dissemination and age difference of chicks in susceptibility to *Salmonella* Pullorum. Proc. Sec. Nat. Livestock Improvement Conference.

Threlfall, E. J., Cahart, H. (1993): Interrelationships between strains of S. Enteritidis. Epidemiol. Infect.111, 1- 8.

Thrusfield, M. W. (1986): Veterinary Epidemiology London: Butterworth and Co. Ltd.,

Waltman, W. D., Horne, A. M., Pirkle, C. (1993): Influence of environment, incubation time on the isolation of *Salmonella*. Avian Dis. 37, 884- 887.

Waltman, W. D., Mallinson, E. T. (1994): Isolation of *Salmonella* from poultry tissues and environmental samples: Nationwide Survey Avian Dis. 39, 45-54.

Waltman, W. M., Ziprin, R. L., Huff, W. E., Barrey, R. B. (1994): Effect of Othratoxin A on *Salmonella*- challenged broiler chicks. Poult. Sci. 1241- 1248.

Watner-Toews, D. (1996): An agroecosystem health perspective on foodborne illness Ecosystem Health 2, 177- 185

WHO. (1992): Antigenic formulas of the *Salmonella* serovars. Taxonomy of the genus *Salmonella*. Changes in serovars nomenclature Geneva : France WHO pp 5.

WHO. (1993): Guidelines for the establishment of systems for the epidemiological surveillance of food-borne diseases and the investigation of outbreaks of food poisoning Geneva 27.

WHO. (1994): Guidelines on detection and monitoring of *Salmonella* infected flocks with particular reference to S. Enteritidis Geneva: WHO (Zoon./ 94. 173)

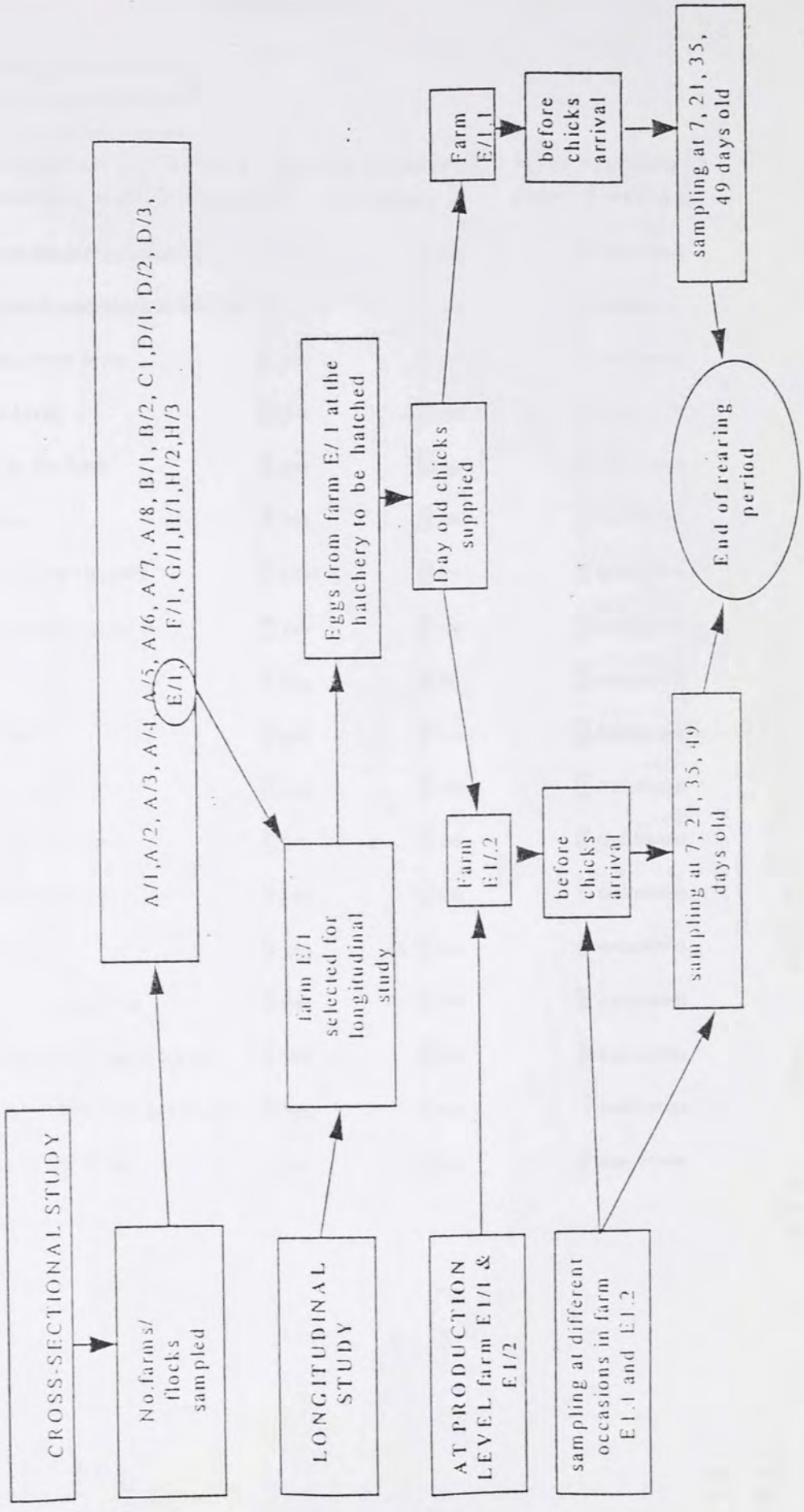
William, J. E., Mallinson, E. T., Snoeyenbos, G. H. (1980): Isolation and identification of avian pathogensThe American Association of Avian Pathologists 2nd ed. pp 1- 8.

Zeidler, G. (1996): How can Foodborne micro-organisms make you ill? Poult. Bull. Feb, 105- 107.

Ziprin, R. L., Corrier, D. E., Deloach, J. R. (1992): Control of established Salmonella Typhimurium intestinal colonization with in vivo-passaged anaerobes. Avian Dis.37, 183- 188.

8. APPENDICES

Appendix 1



Appendix 2

Questionnaire

Date----------/----------/----------
Name of the farm----------0
Ownership----------
Type of production-(broilers ☐ layers ☐ broiler breeders ☐ layer breeders ☐)
Age----------less than week 2-4 weeks ☐ 4-8 weeks ☐ above 8 weeks ☐

Foot and vehicle baths at the farm entrance	☐ yes	☐ no	☐ unknown
Accessibility of wild animals and birds in houses	☐ yes	☐ no	☐ unknown
Free movement of materials in farm	☐ yes	☐ no	☐ unknown
Frequent visitors in the farm	☐ yes	☐ no	☐ unknown
Good hygiene barriers in the farm	☐ yes	☐ no	☐ unknown
Good sanitation condition	☐ yes	☐ no	☐ unknown
Rotation of workers within the houses	☐ yes	☐ no	☐ unknown
Any species other than poultry in farm	☐ yes	☐ no	☐ unknown
Waste deposit in pit	☐ yes	☐ no	☐ unknown
Control of flies and vermin	☐ yes	☐ no	☐ unknown
Any poultry < 1km from the farm	☐ yes	☐ no	☐ unknown
Is the farm surrounded by village	☐ yes	☐ no	☐ unknown
Cleaning and disinfection done properly	☐ yes	☐ no	☐ unknown
Any nutritional deficiency	☐ yes	☐ no	☐ unknown
Are birds generally in good condition	☐ yes	☐ no	☐ unknown
Are houses surrounded by clean environment	☐ yes	☐ no	☐ unknown
Deposit of waste materials <300m from houses	☐ yes	☐ no	☐ unknown
Is there disease problem in the farm	☐ yes	☐ no	☐ unknown

Appendix 2

Disease problem	yes	no	unknown
Any mortality reported	yes	no	unknown
Any salmonellosis prior/during 1996-97	yes	no	unknown
Are birds vaccinated against: Newcastle disease	yes	no	unknown
Marek's disease	yes	no	unknown
Any other	yes	no	unknown
Any routine vaccination	yes	no	unknown
Any disease monitoring	yes	no	unknown
Are vaccines available locally	yes	no	unknown
Any antibiotics are used in farm	yes	no	unknown
Recording system good	yes	no	unknown
Are waste used as manure	yes	no	unknown
Are waste used as food additive	yes	no	unknown
Appropriate protective clothing used	yes	no	unknown
Are birds kept same age in house	yes	no	unknown
Are birds kept same breed in house	yes	no	unknown
Is sexing of birds done	yes	no	unknown
Is litter changed all in all out	yes	no	unknown
Is cost and benefit analysis of farm done	yes	no	unknown
Market available for farm products	yes	no	unknown
Knowledge about poultry management	yes	no	unknown
Knowledge about poultry diseases	yes	no	unknown

Appendix 3

Types and preparation of media

Broth and agar media were used in the process of isolation and were prepared as recommended (ISO, 1993).

Broth

a) **BPW (Merck 1- 07228):** was prepared per liter. For each liter of distilled water, the amount of 25.5g of BPW was added and allowed to dissolve well then. The medium was then dispensed into flasks at the volume of 225ml and sterilized in the autoclave set at 121°C for 20 minutes. The medium was then stored in refrigerator at + 4°C.

b) **RV- broth (Merck 1- 07700)**: For every one liter of distilled water 43g of the media was added then dispensed into universal bottles at the volume of 10ml and autoclaved for 15 minutes at 115°C. The medium was then stored in a refridgerator at + 4°C.

c) **TBG broth (Merck 1- 05285):** 46g of TGB- broth base was suspended in one liter of distilled water and brought to boiling briefly and cooled. Prior to use 20ml iodine/potassium iodine solution and 10ml of a 0.1% brilliant green solution per litter was added.

Preparation of *iodine/potassium iodine*
6g potassium plus 5g iodine were mixed in 20ml distilled water.
0.1% brilliant green solution: 1g brilliant green was added to one liter distilled water and stored in a refridgerator for further use. TGB- broth was prepared same day of use.

d) **Lysine decarboxylation medium**: 5.0g L-Lysine monohydrocloride, 3.0g yeast extract, 1.0g glucose, 0.015g bromocresol purple were dissolved in one liter of distilled water. The pH was adjusted to 6.8. The medium was then poured into test tubes at a quantity of 5ml. Then it was autoclaved for 10 minutes at 121°C. After cooling the medium was placed in a refridgerator at + 4°C.

Agar

a) **BPLS agar (Merck- 10747)**: 52g BPLS agar was dissolved in one liter of distilled water and autoclaved for 15 minutes at 121°C. The medium was then poured in large sized Petri dishes. When cooled the plates were stored in refrigerator.

Appendix 3

Agar

b) **XLD Agar (Merck- 5387)**: 55g of XLD agar was dissolved in one liter insterile distilled water. The medium was completely dissolved in boiling water bath. It was the poured in large sized Petri dishes and stored in refrigerator when cooled for storage.

c) **Nutrient Agar (Merck- 7882):** 28g of the agar was dissolved in one liter of distilled water and autoclaved for 15 minutes at 121°C. It was then poured in petridishes and stored in refrigerator at + 4°C.

d) **TSI agar (Oxoid- CM 277)**: 65g of TSI agar was dissolved in one liter of distilled water, allowed to dissolve completely in boiling water bath. The medium was then dispensed in test tubes at about 5ml volume then autoclaved. The medium was then allowed to set in sloping position to give a butt of 2.5cm depth.

e) **Simmon Citrate Agar Merck- 1. 02501)**: 22.5g was dissolved in one liter of distilled water put in boiling water bath, dispensed in test tubes and finally autoclaved for 15 minutes at 121°C. The agar was then poured in slant agar tubes and allowed to cool.

f) **MAC agar (Oxoid- CM 109)**: A 50g was dissolved in one liter of distilled water and then autoclaved for 15 minutes at 121°C. The media was poured in Petri dishes and allowed to cool before storing.

g) **Gassner Agar (Merck- 1282)**: 77g was dissolved in one liter of distilled water and finally autoclaved for 15 minutes at 121°C. The media was then poured in Petri dishes and allowed to cool.

Appendix 4

Daily layer record

Batch No.---------- Breed:----------Year:----------
Hatch date/ age:---------- Supplier:----------Month:----------
Starting No. birds:---------- Birds m^2----------

Day	Eggs laid	Broken eggs	No. Birds	Dead	Culls/ sales	Chick feed/kg	Vaccination/ treatment	Diagnosis	Remarks
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									
Total									

Chick feed:--------kg at--------per kg Eggs per bird per day:----------
Growers feed:-----kg at--------per kg Feed per bird per day:----------Layers
feed:-------kg at--------per kg Profit (egg value- feed cost):----
Total feed:---------- **Total cost**: **Total for the month**----------

Appendix 4

Production record for broilers

Hatch date:----------Breed:----------
Starting No. birds:--------
Supplier:----------
Batch N.----------

Week	Number of birds	Vaccination Type	Treatment	Diagnosis	Number dead	Remarks
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
Total						

Cost of day old chicks:----------
Total cost of feed:----------
Other costs:----------
Total costs:----------
Value of birds:----------
Profit:----------

Appendix 5

Map of Ethiopia indicating the study area (Debre Zeit)

9. CURRICULUM VITAE

Name:	Dr. (Mrs.) Marosi Augustine Molomo
Profession:	Veterinarian
Nationality:	Lesotho
Rank:	District Veterinary Officer
Date of birth:	12/ 12/ 1963
Languages:	Sotho, English and Spanish
Permanent Address:	P. O Box 1895 Maseru 100 Lesotho. Tel. (266) 323687
Marital status:	Married

Education record:

1970 - 1976	Primary education- Maseru
1977 - 1979	Junior certificate- Roma
1980 - 1981	Higher certificate-Roma
1983 - 1989	Doctor of Veterinary Medicine (DVM), University of Camaguey, Cuba.
1996 - 1997	Master of Science Degree in Tropical Veterinary Medicine, Free University of Berlin, Germany and Addis Ababa University, Ethiopia).

Work experience:

1989 - Veterinary officer under the Ministry of Agriculture, Animal Health Division
1990 - District Veterinary Officer 1990- in Mafeteng to date.

Research experience:

1994 - Involved in the investigation of Lumpy Skin Disease.
1995 - Involved in the investigation of Anthrax outbreak.

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

Name MARGI A. MOLOMO

Signature M.M Shomo

Date of Submission 7th JANUARY, 1998

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

Dr. A. Käsbohrer A. Kaesbohrer

Dr Adene Zerihun A. Zerihun