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THE PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *SALMONELLA* SPECIES FROM EXOTIC CHICKEN EGGS IN ALAGE AND SURROUNDING TOWNS, ETHIOPIA

MSc Thesis

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A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Public Health

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LIST OF ABBREVIATIONS

ACC	Ampcillin, Chioramphenicol and Cotrimoxazole
ACMSF	Advisory Committee on Microbiological Safety of Food
ATVET	Agricultural Technical Vocational Educational Training
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CDC	Centre for Disease Control
CNS	Central Nervous System
CVMA, AAU	College of Veterinary Medicine and Agriculture, Addis Ababa University
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FSRIO	Food Safety Research Information Office
HACCP	Hazard Analysis Critical Control Point
HIV	Human Immuno Deficiency Virus
H_2S	Hydrogen Sulphide
ISO	International Organization for Standardization
LPS	Lipopolysaccharide
MCFA	Medium Chain Fatty Acid
MDR	Multidrug Resistance
MDRST	Multidrug Resistance *Salmonella*Typhi
MOS	Mono Oligosaccharide
MSRV	Modified Semisolid Rappaport Vassiliadis
NPIP	National Poultry Improvement Plan
OIE	Office International Des Epizotic
RES	Reticulo Endothelial System

LIST OF ABBREVIATIONS CONTINUED

RV	Rappaport Vassiliadis
SCFA	Short Chain Fatty Acid
Spp	Species
TSI	Triple Sugar Iron
TVET	Technical Vocational Educational Training
UK	United Kingdom
US	United States
USA	United States of America
VP	Vogues-Proskauer
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate
Y. enterolitica	*Yersina enterolitica*

ABSTRACT

This study was carried out from November 2012 to April 2013 to determine the prevalence and antibiotic susceptibility patterns of *Salmonella*isolated from exotic chicken egg from Alage, Ziway and Shashemene farms. Two samples, from shell and content were taken from a single egg. Then pre-enrichment with buffered peptone water, enrichment with RV broth, plating on XLD and BGA agar were done. After that, positive samples transferred in to nutrient agar and biochemical tests (TSI agar, urea broth, lysine decarboxylation and indole test were done. From 196 eggs a total of 392 samples were taken and 52 (13.3%) were found to be positive of *Salmonella*. From these 52 positive samples 30 (7.7%) were from egg shell and 22 (5.6%) were from egg content.The total prevalence of *Salmonella* varied among the farming systems. The prevalence of *Salmonella*in egg content from semi-intensive farm (10%) was significantly higher than the prevalence of *Salmonella*from intensive farm (3.4%). The prevalence of *Salmonella* in egg shell from semi-intensive farm (11.5%) was also significantly higher than the prevalence of *Salmonella* in egg content from intensive farm (5.7%).The difference in prevalence observed between egg shell (7.7%) and egg content (5.6%) was having statistically significant difference.All the 52 *Salmonella* isolates were subjected to antimicrobial susceptibility test, using elevendifferent antimicrobials. Of these only one isolate was resistant to Ciprofloxacin and Ceftriaxon. Ampicillin is highly resisted (55.8%) followed by Tetracycline (36.5%), Naldixic acid (30.8%) and Sulphamethoxin-trimethoprim (30.8%).

Key words: *Antimicrobial Susceptibility, Egg content, Egg shell, Prevalence, Salmonella.*

1. INTRODUCTION

Salmonellosis is an infectious disease of humans and animals caused by organisms of the two species of *Salmonella* (*Salmonella enterica* and *Salmonella bongori*). Although primarily intestinal bacteria, *Salmonella* is widespread in the environment and commonly found in farm effluents, human sewage and in any material subjected to faecal contamination. *Salmonella* organisms are etiological agents of diarrhoeal and systemic infections in humans, most commonly as secondary contaminants of food originating from animals and the environment, usually as a consequence of subclinical infection in food animals leading to contamination of meat, egg and milk or secondary contamination of fruits and vegetables that have been fertilized or irrigated by faecal wastes. Human salmonellosis is one of the most common and economically important zoonotic diseases (OIE, 2010).

Infections due to *Salmonella* spp. remain a global problem. These infections may cause significant morbidity and mortality both in humans and production animals as well as considerable economic losses. *Salmonella*spp. are typically transmitted among humans and animals via a faecal-oral route, usually through the consumption of contaminated food or water. Timely identification and serotyping of *Salmonella* from clinical specimens facilitates outbreak detection and patient management while prompt and accurate detection of *Salmonella* spp. in contaminated food or water provides an opportunity to prevent the contaminated food from entering the food supply (WHO, 2010).

Chickens can be infected with many different serovars of *Salmonella.* Some serovars, such as *Salmonella*Pullorum and *Salmonella*Gallinarum, are host specific for chickens, whereas other serovars, such as *Salmonella*Typhimurium, *Salmonella*Enteritidis and *Salmonella* Heidelberg are able to infect a wide range of hosts. There are a number of commonly identified types of *Salmonella* associated with chickens with the most

common serovars being *Salmonella* Enteritidis, *Salmonella* Kentucky, *Salmonella* Heidelberg (CDC, 2006).

Eggs and egg products are nutritious foods and they form an important part of the human diet. Consuming contaminated egg, however, has been associated with negative health impacts. Eggs and egg products that are improperly handled can be a source of foodborne diseases, such as salmonellosis (FAO, 2002).

Salmonella is a rod shaped motile, aerobic and facultative anaerobe, non-spore forming and Gram negative organism. It can grow from 5°C up to 47°C, with an optimum temperature of 37°C. *Salmonella* is heat sensitive and can be readily destroyed at pasteurization temperature. *Salmonella* is a general name used for a group of more than 2,400 closely related bacteria that cause illness by reproducing in the digestive tract. Each *Salmonella* serotype shares common antigens but own name. *Salmonella* Enteritidis was the commonest serotype isolated from human clinical specimens (D'Aoust, 2000).

Foodborne diseases caused by *Salmonella* serotypes occur at high frequency in industrialized nations and developing countries and represent an important public health problem worldwide. Most of the *Salmonella* infections in humans resulted from the ingestion of contaminated poultry product and these infections are associated with the consumption of raw eggs and foods containing raw eggs (Carli*et al*., 2001).

Until the mid 1980's the first line drugs like Ampicillin, Chloramphenicol and Co-trimoxazole (ACC) were used as standard treatment for enteric fever. Simultaneous resistance to three or more different groups of antimicrobial drugs is defined as MDR *Salmonella*. MDR *Salmonella* Typhi has appeared throughout the world, especially in South America, the Indian sub continent, Africa and also in South East Asia. In India,

South America, the Indian sub continent, Africa and South East Asia. In India, *Salmonella* drug resistance has been reported since 1960 following the first outbreak of MDR *Salmonella* in Calicut. MDR *Salmonella* is still common in many areas, although in some regions highly sensitive strains have re-emerged (Gopal *et al*., 2011).

Despite some attempts to study prevalence of *Salmonella* in egg's content in Ethiopia, mainly it was mixed thoroughly with poultry and beef meat. The status of the *Salmonella* in chicken table egg in many parts of Ethiopia is not well known. However, studies made elsewhere indicated that chicken eggs are important sources of *Salmonella* particularly among those raw consumers due to egg shell contamination. Moreover, none of the previous studies in Ethiopia on chicken table eggs determined the occurrence, magnitude and distribution of *Salmonella* in both exotic as well as local chicken table eggs (Minte *et al*., 2011).

There are a few reports on the prevalence *Salmonella* from egg in Ethiopia. Thus the current study was designed to provide valuable information as to the extent of *Salmonella* in eggs of poultry from different farms of exotic chicken from Alage, Ziway and Shashemene.

Therefore the objectives of this research were :-

- ✓ To determine the prevalence of *Salmonella* from exotic chicken egg content and shell.
- ✓ To determine antibiotic susceptibility pattern of *Salmonella* isolates.

2. LITERATURE REVIEW

2.1. Historical Background

Salmonellosis is one of the most frequently reported foodborne illnesses worldwide. It was originally discovered and named after Dr. Daniel Salmon over 100 years ago. *Salmonella* spp. infects a wide range of hosts including humans and can cause diseases ranging from severe enteric fever to self-limiting gastroenteritis that, in some individuals, can become systemic and life-threatening. These microbes are among the most ubiquitous organisms that cause bacterial diarrhoea and can cause paratyphoid or typhoid fever, depending upon the strain (Lacey, 1993).

In the early 1900s, pullorum disease and fowl typhoid, caused by *Salmonella* Pullorum and *Salmonella* Gallinarum, respectively, were widespread in the United States (Shivaprasad, 2003). The National Poultry Improvement Plan established in 1935, was partly designed to help control and eradicate pullorum disease and fowl typhoid. Pullorum disease and fowl typhoid were eradicated from commercial flocks largely through control programs, such as NPIP, by the mid 1960s. It has been proposed that the emergence of *Salmonella* Enteritidis infections in the 1990s may correspond with the eradication of *Salmonella* Gallinarum in poultry. Prior to the 1960s, *Salmonella* Enteritidis was rare among poultry; however, with the reduction and elimination of *Salmonella* Gallinarum and *Salmonella* Pullorum, the prevalence of *Salmonella* Enteritidis in chickens increased. Before the increase in *Salmonella* Enteritidis infections in chickens, the serovar was commonly detected in rodents and potentially made the jump to birds as immunity to *Salmonella* Pullorum waned in the flocks (Baumler *et al*., 2000).

The prevalence of *Salmonella* on egg shell has not yet been fully investigated. The microbiological quality of eggshell influences the quality of the egg products. Eggshell can become contaminated with *Salmonella* either because of an infection of the oviduct

or by environmental contamination due to the shedding of the bacteria by infected animals (Little, 2005).

Almost 80% of the cases and deaths are in Asia and the rest occur mostly in Africa and Latin America. It is estimated that there are 22 million new cases of enteric fever annually, with 200,000 deaths. Regions with the highest incidence of enteric fever (100 cases per 100,000 persons per year) are South Central Asia and South East Asia. Regions of moderate incidence (10-100 cases per 100,000 persons per year) include the rest of Asia, Africa, Latin America and the Caribbean and Oceania, except for Australia and New Zealand. In Delhi, India, the incidence of enteric fever is 9.8 cases per 1,000 people per year. *Salmonella enteric* serovar Typhiand Paratyphi A are the predominant types of etiological agents responsible for enteric fever in India, particularly during summer (Kavita *et al*., 2009).

2.2. Classification and Nomenclature

According to the latest nomenclature, which reflects recent advances in taxonomy,the genus *Salmonella* consists of only two major species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is divided into six subspecies, which are distinguishable by biochemical characteristics and susceptibility to lysis by bacteriophage. These subspecies are: subspecies enterica, subspecies salamae, subspecies arizonae, subspecies diarizonae, subspecies houtenae, subspecies indica (Grimont& Weill, 2007).

Historically *Salmonella* had been named based on the original places of isolation such as *Salmonella* London and *Salmonella* Indiana. This nomenclature system was replaced by the classification based on the susceptibility of isolates to different selected bacteriophages which is also known as phage typing. Phage typing is generally employed

when the origin and characteristic of an outbreak must be determined by differentiating the isolates of the same serotype. It is very reproducible when international standard sets of typing phages are used (Bhunia, 2008). More than 200 definitive phage types have been reported so far. For example, *Salmonella* Typhimurium DT104 designates a particular phage type for Typhimurium isolates (Hanes, 2003; Andrews and Baumler, 2000).

Epidemiologic classification of *Salmonella* is based on the host preferences. The first group includes host restricted serotypes that infect only humans such as *Salmonella* Typhi. The second group includes host adapted serotypes which are associated with one host species but can cause disease in other hosts such as *Salmonella* Pullorum in avian. The third group includes the remaining serotypes. Typically, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg are the three most frequent serotypes recovered from humans each year (Boyen *et al*., 2008).

All *Salmonella* strains are serologically classified using Kauffmann-White scheme, and at the present the genus contains more than 2,500 serotypes. The majority of the *Salmonella* serotypes belong to *Salmonella enteric* subspp. *enterica* (about 60%), followed by subspecies salamae (20%), diarizonae (13 %), arizonae (3.8 %), houtenae (2.8%) and indica (0.45%). Only (0.8%) belong to the second species *Salmonella bongori* (Popoff *et al*., 2003).

2.3. General Characteristics of *Salmonella*

Salmonella is a rod-shaped, motile, aerobic and facultatively anaerobic, non-spore forming and Gram negative organism. It can grow from 5°C up to 47°C with an optimum at 37°C. *Salmonella* is heat sensitive and can be readily destroyed at pasteurization temperature. The infectious dose is usually greater than 10^2 to 10^3organisms and may

vary with age and health status of the host. In some cases, it can be as few as 15 to 20 cells (D'Aoust, 2000).

Salmonella is typically motile by means of peritrichious flagella; however, non motile variants may be encountered and the host adapted avian pathogens like *Salmonella* serotype Pullorum and Gallinarum are always nonmotile (WHO, 2010).

2.4. Colony Characteristics

Typical colonies of *Salmonella* on BGA are pink and cause the colour of medium to change to red. Typical colonies of *Salmonella* grown on XLD agar have a black centre and a lightly transparent zone of reddish colour due to the colour change of the indicator. H_2S negative variants grown on XLD agar are pink with a darker pink centre. Lactose-positive *Salmonella* grown on XLD agar are yellow with or without blackening (Boyen *et al*., 2008).

2.5. Biochemical Properties

As is typical of all other *Enterobacteriaceae*, the *Salmonellae* are Gram-negative, oxidase negative, facultative anaerobes. The *Salmonellae* are Vogues-Proskauer (VP) negative, methyl red positive, and reduce nitrate to nitrite without the production of gas. The *Salmonellae* are typically indol and urease negative, although rare indol or urease positive strains may be encountered (WHO, 2010).

2.6. Isolation and Identification

2.6.1. Identification and detection of Salmonella

Diagnosis is based on the isolation of the organism either from tissues collected aseptically at necropsy or from faeces, rectal swabs or environmental samples, food products. *Salmonella* may be isolated using a variety of techniques that may include pre-enrichment to resuscitate sublethally damaged *Salmonella*, enrichment media that contain inhibitory substances to suppress competing organisms, and selective plating agars to differentiate *salmonellae* from other enterobacteria. Various biochemical, serological and molecular tests can be applied to the pure culture to provide a definitive confirmation of an isolated strain (OIE, 2010).

Culture

There are numerous methods for isolation of *Salmonella* in use worldwide. The culture techniques and media that may work best in a particular diagnostic situation depend on a variety of factors, including the *Salmonella* serovar, source and type of specimens, animal species of origin, experience of the microbiologist and availability of selective enrichment and selective plating media. All culture media should be subjected to quality control and must support growth of the suspect organism from a small inoculum in the presence of a relevant sample matrix. The core of the standard method is pre-enrichment in buffered peptone water, enrichment on modified semi-solid Rappaport–Vassiliadis (MSRV) and isolation on xylose-lysine-deoxycholate (XLD) and an additional plate medium of choice (Reissbrodt, 1995).

Biochemical test

Conventional biochemical testing is typically used to differentiate the genus *Salmonella* from other *Enterobacteriaceae*, to differentiate between the six subspecies of *Salmonella enterica* and to differentiate *Salmonella enterica* from *Salmonella bongori*. With limited exceptions, *Salmonella* serovars cannot be differentiated from each other on the basis of

biochemical profile. Biochemical identification becomes an essential supplement to serotype data when multiple subspecies share an identical antigenic formula, or when all antigenic factors are not expressed, such as with non-motile, mucoid, or rough isolates (Mikoleit, 2010).

2.7. Epidemiology of *Salmonella*

A peculiar epidemiological feature of human salmonellosis is that epidemics are commonly associated with a particular prevalent serovar of *Salmonella enterica* that shows temporal and geographical variation. Until the 1980s, *Salmonella enteric* serovar Typhimurium (*Salmonella* Typhimurium) was the serovar most commonly isolated from humans worldwide but by the late 1980s, *Salmonella enteric* serovar Enteritidis (*Salmonella* Enteritidis) emerged as the most common cause of salmonellosis in Europe, and during the 1990s, it became the most prevalent serovar in many countries worldwide (Mishu, 1994).

The reasons for this worldwide serovar shift are still not understood, and several hypotheses have been proposed, including the existence of a rodent reservoir for *Salmonella* Enteritidis or the epidemiological change induced by vaccination of poultry against the closely related bacterium *Salmonella* Gallinarum (Ward *et al*., 2000).

2.7.1. Salmonella infection in human and sources of transmission

Human salmonellosis is usually contracted from the consumption of undercooked meat and poultry, raw eggs and milk. Yet, many other foods have been implicated in the spread of *Salmonella* organisms. Many times this illness occurs because of in-home or consumer contamination, not because the product was tainted during processing. Although consumers may argue that illness would not have occurred if the product was not initially

contaminated. It is important to take into account food preparation surface cleanliness and personal hygiene practices, as they play key roles in the transfer of bacteria. This is just one of the reasons that education on proper handling and cooking procedures is so vital in consumer safety. In developed countries, human to human transfer is uncommon but can occur (FSRIO, 2005).

2.7.2. Salmonella transmission in poultry

In vertical transmission, *Salmonella* are introduced from infected reproductive tissues to eggs prior to shell formation. *Salmonella* serotypes associated with poultry reproductive tissues that are of public health concern include *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg. Among the different serotypes, *Salmonella* Enteritidis may be better able to achieve invasion and as a consequence, may be found more frequently in reproductive tissues. Horizontal transmission is usually derived from faecal contamination on the egg shell. It also includes contamination through environmental vectors, such as farmers, pets and rodents. Many different serotypes of the genus *Salmonella* can be involved. They may be able to contaminate egg contents by migration through the egg shell and membranes. Such a route is facilitated by moist egg shells, storage at ambient temperature and shell damage (ACMSF, 2001).

Backyard hens can also be infected through contact with wild animals, domestic mammals and commercial poultry that are carriers of *Salmonella* and consequently may play a role in the transmission of the organism to other animals and humans (Jafari *et al*., 2007). In addition, *Salmonella* Enteritidis infection in chickens is often silent with no evident morbidity or mortality among infected chickens so there may not be any outward indication of *Salmonella* Enteritidis infection among chickens in farm flocks or in the eggs they produce (Gast *et al*., 2005).

2.7.3. Transmission vehicles

Salmonella are widely distributed in nature and they survive well in a variety of foods. Poultry eggs and dairy products are the most common vehicles of salmonellosis. In recent years, fresh produce like fruits and vegetables have gained concern as vehicles of transmission where contamination can occur at multiple steps along the food chain (Bouchrif *et al*., 2009).

First, environment contaminated with *Salmonella* serves as the infection source because *Salmonella* can survive in the environment for a long time. After that, *Salmonella* is transmitted to vectors such as rats, flies and birds where *Salmonella* can shed in their faeces for weeks and even months. Following the direct transmission, moving animals such as swine, cows and chickens act as the important risk factor for infection. These animal reservoirs are infected orally because *Salmonella* normally originates from the contaminated environment and also contaminated feed. Human get infected when eating the food or drinking the water that is contaminated with *Salmonella* through animal reservoirs. However, *Salmonella* Typhi and *Salmonella* Paratyphi A do not have animal reservoir, therefore infection can be happened by eating the improperly handled food by infected individuals (Newell *et al*., 2010).

Besides, transmission of *Salmonella* to the food processing plants and equipments for food preparation are also of great importance. Once carried by vectors or transferred to food, consumption by human can result in the risk of salmonellosis. The *Salmonella* cells can attach to food contact surfaces such as plastic cutting board which may develop into biofilm once attached and hence cause cross-contamination. Consequently, *Salmonella* can enter the food chain at any point from livestock feed, through food manufacturing, processing and retailing as well as catering and food preparation in the home (Wong *et al*., 2002).

2.8. Virulence Factors and Pathogenesis

Although many aspects of the pathogenesis of salmonellosis are poorly understood, particularly the relationship between *Salmonella* toxin and cell damage, some of the general features associated with virulence are known. The virulence of *Salmonella* relates to their ability to invade host cells, replicates in them and resist both digestions by phagocytes and destruction by the complement components of plasma. Following adherence, probably through fimbrial attachment, to the surface of intestinal mucosal cells, the bacteria induce ruffling of cell membranes. The ruffles facilitate uptake of the bacteria in membrane bound vesicles, which often coalesce. The organisms replicate in these vesicles and are eventually released from the cells, which sustain only mild or transient damage. The complex invasion process is mediated by the products of a number of chromosomal genes, whereas growth within host cells depends on the presence of virulence plasmids (Quinn *et al*., 2002).

Resistance to digestion phagocytes and to the lethal action of complement components facilitates the spread of organisms within the host. The toxic oxidative effects of free radicals produced by phagocytes are minimized by bacterial catalase and superoxide dismutase activities. Resistance to killing by complement is partially dependent on the length of O antigen chains of lipopolysaccharide (LPS). Long chains of LPS prevent the complement components of the membrane attack complex from interacting with and damaging the bacterial cell membrane. The LPS is also responsible for the endotoxic effect of infection with *Salmonella*. It may contribute to the local inflammatory response which damages intestinal epithelial cells and results in the development of diarrhoea. Bacterial cell wall LPS also mediates the endotoxic shock which may accompany septicemic salmonellosis (Bhunia, 2008; Quinn *et al*., 2002).

2.9. Clinical Features

Generally speaking, the infectious dose, incubation period, symptoms and mode of transmission of salmonellosis caused by different serotypes are similar. Symptoms include diarrhoea, fever and abdominal cramps with incubation period ranges from 12 to 72 hours. The illness usually last 4 to 7 days and most people recover without treatment. The elderly, infants and those with impaired immune systems are more likely to have a severe illness. Some specific serotypes like *Salmonella* Typhi and *Salmonella* Paratyphi are also foodborne pathogens causing a systemic illness called typhoid fever and paratyphoid fever respectively. Their spread is predominantly by food and water contaminated by faeces of patients and carriers (Gray and Fedorka, 2002).

2.9.1. In animals

Salmonella species are often carried asymptomatically. Clinical disease usually appears when animals are stressed by factors such as transportation, crowding, food deprivation, weaning, parturition, exposure to cold, a concurrent viral or parasitic disease, sudden change of feed or overfeeding following a fast. Salmonellosis is common in horses after major surgery. In some cases, oral antibiotics may also precipitate disease (Panisello *et al*., 2000).

The clinical signs vary with the infecting dose, health of the host, *Salmonella* serovar and strain, and other factors. Some serovars tend to produce a particular syndrome: for example, in pigs *Salmonella* Choleraesuis is usually associated with septicemia and *Salmonella* Typhimurium with enteric disease. Although salmonellosis can be seen in all domestic animals, pregnant, lactating or young mammals and birds are the most susceptible (Gray and Fedorka, 2002).

Ruminants, pigs and horses

The major syndromes in livestock are enteritis and septicemia. Acute enteritis is the most common form in adult animals and in calves over a week old. This form is characterized by profuse diarrhea, dehydration, depression, abdominal pain and anorexia. The faeces are watery to pasty, often foul smelling, and may contain mucus, pieces of mucous membrane, casts or blood. A fever occurs early in the infection, but can disappear by the time diarrhoea develops. In dairy cows, milk production drops acutely (Gray and Fedorka, 2002).

Intestinal salmonellosis usually lasts for 2 to 7 days. Death can occur as the result of dehydration and toxemia. Horses, in particular, often have severe enteritis and may die within 24 to 48 hours. Loss of condition, emaciation and unthriftiness may be seen in surviving livestock. Recovery can be slow. Subacute enteritis may be seen in adult horses, cattle and sheep. The most obvious symptoms are persistent soft feces or diarrhea, and weight loss. There may also be mild fever, inappetence and some dehydration (Panisello *et al*., 2000).

Chronic enteritis is mainly seen in older calves, adult cattle and growing pigs. The symptoms can include progressive emaciation, low grade intermittent fever and inappetence. The feces are usually scant and may be normal or contain mucus, casts or blood. Rectal strictures can be sequelae in growing pigs. Septicemia is the most common syndrome in very young calves, lambs and foals, and in pigs up to 6 months of age. The symptoms include marked depression, high fever and, often, death within 1 to 2 days. Diarrhea can occur in some animals. Central nervous system (CNS) signs or pneumonia may be seen in calves and pigs. Pigs may also develop a dark reddish or purple discoloration of the skin, particularly on the ears and ventral abdomen (Gray and Fedorka, 2002).

Pregnant animals may abort, either with or without other clinical signs. Serovars often associated with abortions include *Salmonella* Dublin in cattle, *Salmonella* Abortusovis in sheep and *Salmonella* Abortusequi in horses. In cows with subacute enteritis, the first symptom may be abortion, followed after several days by diarrhea. Abortions in pregnant ewes may be followed by a foetid, dark red vaginal discharge and sometimes death (Panisello *et al*., 2000).

Dogs and cats

In dogs and cats, the most common form is acute diarrhea with or without septicemia. Most cats and dogs with acute diarrhea recover within 3 to 4 weeks. Pneumonia, abscesses, meningitis, osteomyelitis, cellulitis or conjunctivitis may also be seen. A chronic febrile illness characterized by anorexia and lethargy, but no diarrhea, has been reported in cats. Pregnant dogs and cats may abort or give birth to weak puppies or kittens (EFSA, 2006).

Birds

Most clinical cases are seen in very young birds. The symptoms may include anorexia, lethargy, diarrhea, increased thirst and CNS signs (EFSA, 2006).

2.9.2. In humans

In human disease, the clinical pattern of salmonellosis can be divided into five disease patterns namely enteric fever, gastroenteritis, bacteremia and other complications of nontyphoidal salmonellosis as well as chronic carrier state (Parry, 2006).

The annual number of *Salmonella* infections in humans is tremendously high worldwide. A worldwide egg associated salmonellosis pandemic has started in the 1970s and is currently fading away because of huge efforts of policy makers and the poultry industry. This pandemic has been caused by the serotype *Salmonella* Enteritidis. Due to its preferential association with hen eggs, combined with the way humans tend to store (room temperature), handle and eat (uncooked eggs), *Salmonella* Enteritidis had and still has a major impact on human health. While total European Union *Salmonella* contamination levels are decreasing in recent years, the antimicrobial resistance of the *Salmonella* isolates is still increasing. Especially serotype Typhimurium is causing concerns, as about 40% of all *Salmonella* Typhimurium strains isolated in 2006 were resistant to 4 or more antimicrobials (Van Immerseel *et al*., 2005).

Enteric fever

Salmonella Typhi causes typhoid fever whereas Paratyphi A, B and C cause paratyphoid fever with symptoms which are milder and a mortality rate that is lower for the latter. Both serotypes are solely human pathogens. Infection typically occurs due to ingestion of food or water contaminated with human waste. In recent years, antibiotic resistant strains have been isolated in most endemic areas, particularly South East Asia, India, Pakistan and Middle East (Scherer and Miller, 2001).

Roughly 10% of patients may relapse, die or encounter serious complications such as typhoid encephalopathy, gastrointestinal bleeding and intestinal perforation. Relapse is the most common occurrence probably due to persisting organisms within reticuloendothelial system (RES). Typhoid encephalopathy, often accompanied by shock, is associated with high mortality. Slight gastrointestinal bleeding can be resolved without blood transfusion but in 1 to 2% of cases can be fatal if a large vessel is involved. Intestinal perforation may present with abdominal pain, rising pulse and falling blood

pressure in sick people. Hence, it is very serious in 1 to 3% of hospitalized patients (Hu and Kopecko, 2003; Parry, 2006).

Gastroenteritis

Nontyphoidal salmonellosis or enterocolitis is caused by at least 150 *Salmonella* serotypes with *Salmonella* Typhimurium and *Salmonella* Enteritidis being the most common serotypes in the world. Infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste (Gray and Fedorka, 2002; Yousef and Carlstrom, 2003).

Bacteremia and other complications of nontyphoidal salmonellosis

About 8% of the untreated cases of salmonellosis result in bacteremia. Bacteremia is a serious condition in which bacteria enter the bloodstream after passing through the intestinal barrier. It has been associated with highly invasive serotypes like Cholearaesuis or Dublin. Bacteremia caused by *Salmonella* should be taken into account in cases of fever of unknown origin. Patients with bacteremia and other complications should be treated with antibiotics (Scherer and Miller, 2001; Hanes, 2003).

Chronic carrier state

Salmonellosis can be spread by chronic carriers who potentially infect many individuals, especially those who work in food related industries. Factors contributing to the chronic carrier state have not been fully explained. On average, nontyphoidal serotypes persist in

the gastrointestinal tract from 6 weeks to 3 months, depending on the serotypes. Only about 0.1% of nontyphoidal *Salmonella* cases are shed in stool samples for periods exceeding 1 year. About 2 to 5% of untreated typhoid infections result in a chronic carrier state. Up to 10% of untreated convalescent typhoid cases will excrete *Salmonella* Typhi in feces for 1 to 3 months and between 1 and 4% become chronic carriers excreting the microorganism for more than one year (Scherer and Miller, 2001; Parry, 2006).

2.10. Treatment

Antibiotic treatment should be based on results of susceptibility testing because plasmids coding for multiple resistance are comparatively common in *Salmonellae*. Oral antimicrobial therapy should be used judiciously for treating enteric salmonellosis because it may disturb the normal intestinal flora, extend the duration of *Salmonella* excretion and increase the probability of drug resistance developing. In septicemic form of the disease, intravenous antibiotic therapy must be used. Fluid and electrolyte replacement is required to counteract dehydration and shock (Quinn *et al*., 2002).

2.11. Antimicrobial Resistance

The emergence of antimicrobial resistance, especially the multidrug resistance to Ampicillin, Chloramphenicol and Cotrimoxazole, has further complicated the treatment and management of enteric fever (Mourad *et al*., 2003).

In 1948 Chloramphenicol was introduced to treat the disease. Drug resistant *Salmonella* Typhi has been reported as early as 1972 in Mexico and been obsevered in other countries like Bangladesh, Thailand, Vietnam, Korea, Peru and India. Ampicillin and Cotrimoxazole were effective alternatives drugs till the end of 1990’s when strains

resistant to all the first line drugs used against *Salmonella* at that time, were reported (Lakshmi *et al*., 2006).

Multidrug resistant *Salmonella* Typhi (MDRST) is epidemiologically defined as strains resistant to any two antimicrobials in vitro even if the antimicrobials tested are known to be clinically ineffective. A more useful definition of MDRST is reserved for strains resistant to all three first line antityphoidal antimicrobial agents, namely Ampicillin, Chloramphenicol and Cotrimoxazole. Typhoid fever, caused by MDRST, has become a significant cause of morbidity and mortality over recent years. The incidence of MDRST is reported to be as high as 60%, although there are some reports noting its decline (Muhammad, 2006).

In early study the isolates of *Salmonella* from food items and personnel from Addis Ababa were resistant to the commonly used antibiotics including Streptomycin, Ampicillin, and Tetracycline. The result of the current research also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including Ampicillin, Streptomycin, Nitrofurantoine, Kanamycine and Tetracycline, with resistancerate of 100%, 66.7%, 58.3% and 33.3%, respectively (Zelalem *et al*., 2011).

Ampicillin and Cotrimoxazole, being cost effective and well tried primary drug of choice and Chloramphenicol were the first line drugs used as standard treatment for enteric fever until 1980 (Gopal *et al*., 2011). These first line drugs are abbreviated as ACC: Ampicillin, Cotrimoxazole and Chloramphenicol. The simultaneous resistance to three or more different groups of antimicrobials is defined as multidrug resistance (MDR). Contributing factors to MDR may be drug misuse, drug overuse and inappropriate prescribing practices (Gautam *et al*., 2002).

Invitro experiments showed that fluoroquinolones had higher sensitivity and more effective clinical outcome against *Salmonella* than the ACC (Pegues *et al*., 2005). Since

then, fluoroquinolones (Ciprofloxacin and Ofloxacin) are used as first line drugs instead of the ACC. It should be noted that high resistance of *Salmonella* to Nalidixic acid has been observed as 72.2 % rate (Gopal *et al*., 2011).

2.12. Prevention and Control

Salmonella prevention and control may be achieved by adopting Good Agricultural Practices and Hazard Analysis Critical Control Point (HACCP), and general measures on hygiene and biosecurity procedures in poultry production, in combination with the additional measures, where appropriate. No single measure used alone will achieve effective in *Salmonella* control (OIE, 2010).

Vaccination

Vaccination can be done using live or inactivated vaccines, and vaccines should reduce or prevent the intestinal colonization resulting in reduced faecal shedding and thus egg shell contamination and prevent systemic infection resulting in a decreased colonization of the reproductive tissues, in this way reducing internal egg contamination. It is very well documented that both killed and live vaccines can reduce shedding of *Salmonella* in poultry (Van Immerseel *et al*., 2005).

Recently multiple scientific groups have reported a phenomenon, in which oral administration of *Salmonella* wild type and attenuated strains can confer resistance to infection by a virulent *Salmonella* challenge strain within 24 hr. of administration. This ‘competitive exclusion’ like phenomenon is called colonization-inhibition (Van Immerseel *et al*., 2005; Bohez *et al*., 2008).

It might be possible to administer to newly hatched chicks live *Salmonella* vaccine strains such that they would colonize the gut extensively and very rapidly, inducing a profound resistance to colonization by other *Salmonella* strains of epidemiological significance, which may be present in the poultry house or may also have arisen from the hatchery. Colonization of the gut by the colonization-inhibition strains (live vaccines strains) would prevent gut colonization by virulent strains, while invasion in the gut tissue would evoke an inflammatory response that would prevent invasion to the internal organs by virulent strains(Van Immerseel *et al*., 2005).

Hygiene

Good farming and hygienic practices need to be implemented, in order to avoid introduction of *Salmonella* on the farm or reduce the infection pressure when *Salmonella* is present. Hygienic measures at all levels of the production chain pre-harvest (during life), harvest (catching and transport) and post-harvest are essential for successful *Salmonella* control. Hygienic measures should take into account feed, birds, drinking water, environment, management, cleaning and disinfection. This can imply physical and chemical decontamination treatments of feed, drinking water, the environment of the birds (Graham, 2005).

Use of resistant chicken

The use of a genetically more resistant chicken line might help to control *Salmonella*, although it remains difficult to reconcile selection for disease resistance and selection for performance at the same time because when resistance to disease is maintained production performance may decrease (Sadeyen *et al*., 2004).

Use of probiotics

Probiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health. In this definition it is understood that certain bacterial species multiplying in the colon can have a beneficial effect on host health. These bacterial species can also be administered through the feed. Most probiotics are carbohydrates. Most well known probiotic products added to poultry feed are monooligosaccharides (MOS), glucans, fructo-oligosaccharides and guar gum (Tuohy *et al*., 2005).

Acidification

Acidic compounds are more and more used to combat *Salmonella* infections. Not only drinking water acidification, but also acid release in the proximal gastrointestinal tract (powder as feed additive) or the distal parts of the gastrointestinal tract (coated or encapsulated acids in feed) is widely used. Medium chain fatty acids (MCFA) are strongly bactericidal towards many Gram-positive and Gram-negative bacteria, including *Salmonella* (Nakai and Siebert, 2003). Even at concentrations as low as 10 milli mole MCFA still show a bacteriostatic effect on *Salmonella* (Van Immerseel *et al*., 2004).

Short chain fatty acids (SCFA) are the major bacterial fermentation products in the large intestine. SCFA are also commonly added to feed and drinking water. At high concentrations (1%) these products have an antimicrobial effect in moist environment. This microbial growth inhibition is traditionally explained by the ability of these acids to pass across the bacterial cell membrane in undissociated form, dissociate in the neutral at

bacterial cell interior and thereby acidify the bacterial cell cytoplasm (Tuohy*et al*., 2005).

2.13. Economic Impact of *Salmonella*

Poultry occupies a very crucial part of our economy for being affordable, easily manageable and fast growing compared with other species of animals that provides people with animal protein. The total poultry population of Ethiopia is estimated as 56.5 million, which represents 60% of the total chicken population in East Africa. From the total population of chicken in Ethiopia, 99% are raised under the traditional backyard system of management, while 1% is under intensive management system (Tadelle *et al*., 2003; Ashenafi and Eshetu, 2004).

It is quite evident that poultry farms are flourishing today but in the past mostly extensive type of production was predominating because the major part of poultry production was occupied by individual farmers and consequently, the outcome as a whole was below expectation and limited. Among the factors that played an important role in this regard are poor husbandry practices, low productive breed of the birds and various viral and bacterial avian diseases. Newcastle disease, Marek's disease, Infectious bursal disease, Fowl typhoid, Pullorum disease and Fowl cholera are the most economically important poultry diseases (Tadesse *et al*., 2005).

If an exporting breeding flock is involved, the trading operations for this holding will be suspended until the premises have been depopulated, effectively cleaned/disinfected and replacement flocks have shown to be free of infection. This could mean a loss of trade for several months for the affected holding, disease transmission. Importing and exporting countries agreement and the effect and/ or longer if recurrent infection is not prevented. Fowl typhoid (*Salmonella* Gallinarum) and Pullorum disease (*Salmonella* Pullorum) can cause substantial losses to the poultry farms, sometimes leading to their closure due to the

high cost of eradication on large, complex poultry units. Veterinarians and flock owners or managers should consider *Salmonella* Typhi as the cause of significant mortality in poultry flocks with *Salmonella* Gallinarum especially affecting adult chickens and *Salmonella* Pullorum in young birds (OIE, 2006).

Costs of foodborne bacterial disease were estimated at 44 billion US dollar for 1987 in the United States; salmonellosis accounted for 1.4 billion US dollar of the total and was the largest single cause although *Staphylococcus* food poisoning and *Campylobacter* infection also accounted for about 1 billion US dollar each (Rene and Jane, 2004). Both of these studies sought to estimate costs associated with medical care and lost productivity , productivity losses due to deaths which was a major component of the costs. Other costs were excluded and the amounts stated therefore represented the minimum costs of disease. Comparable estimates of the annual costs of salmonellosis were also reported 4 billion dollar for the United States and 846 million dollar for Canada, although the range of cost categories included in these studies was wider (Tadelle *et al*., 2003).

2.14. Status of *Salmonella* in Ethiopia

In 1972 statistics were reported for the years 1959 to 1963 from the anti-epidemic service. During the five year period an average of 3,469 cases of salmonellosis were reported per annum (17.3 cases per 100,000 inhabitants). It was noted that cases of enteric fever, diagnosed clinically as typhoid fever, were almost three times the number of that of the other types of salmonellosis. This suggests a typhoid feverrate of approximately 4/100,000 inhabitants in the early 1960s. However, the diagnosis of typhoid fever was on clinical grounds and the only microbiological study at the time reported that less than 1% of over 700 stool specimens grew *Salmonella* Typhi (Beyene *et al*., 2008).

It is possible that either the clinical diagnosis of typhoid fever was not accurate or that the methods used for stool analysis did not detect *Salmonella* Typhi from either carriers or cases. It was not until 1981 that a comprehensive study on invasive *Salmonella* in Ethiopia was conducted (Gedebou and Tassew, 1981).

From 1974 to 1981, Gebreyes conducted a study to identify the prevalent serovars and their susceptibility pattern to antibiotics in Addis Ababa. This study serves as a baseline data for all subsequent surveillance studies in Ethiopia. *Salmonella* strains were isolated from adult patients referred to the Central Laboratory and Research Institute, Addis Ababa, between January, 1974 and October, 1981. Of 216 *Salmonella* isolates studied, 54.6% were from stool and 45.4% from invasive sites: blood 34.7%, pus 5.6%, and urine 5.1%. There were 26 different serovars, of which *Salmonella* Typhimurium (48.6%) was the most common (Zinabu *et al*., 2013).

In 1985, Ashenafi and Gedebou reported a study from 1982-1983 to determine the etiology of diarrhoea in adult out-patients in Addis Ababa. A total of 1,000 adult diarrhoeal cases from different hospitals and clinics were investigated and 45 *Salmonella* strains were isolated in the order of prevalence: group C, group B, *Salmonella* Typhi, other group D, group A, and group E. This again raises the possibility that *Salmonella* Concord is the most common cause of gastrointestinal salmonellosis and shows that *Salmonella* Typhi was present (Beyene *et al*., 2008).

In a study conducted in a rehabilitation camp in Korem, a total of 42 (21.1%) of the camp residents had a stool positive culture of *Enterobacteriaceae*, of which only 2% were *Salmonella* species. These were not examined further. There is little information in this study but there is a suggestion of a low level of typhoid carriage in this camp population (Beyene *et al*., 2008).

Between February 1992 and January 1993 a similar study was conducted in Tikur Anbessa Hospital, Addis Ababa, to determine the prevalence of enteric pathogens (*Campylobacter*, *Salmonella* and *Shigella* spp, *Y. enterocolitica* and Enteropathogenic *Escerchia coli*) in 630 adult patients with diarrhoea and 220 patients without diarrhoea. The prevalence of *Salmonella* spp. was 3.8% and 5.9% in patients with diarrhoea and without diarrhoea respectively. This patient group may represent the population more closely than that of the camp in Korem and given the level of isolation of *Salmonella* from non-diarrhoea patients suggests the existence of fairly high numbers of carriers of *Salmonella*, including *Salmonella* Typhi, or undiagnosed typhoid cases. This is in agreement with some previous studies in Ethiopia (Aseffa *et al*., 1997).

Salmonellosis is particularly common in children of developing countries and Ethiopia is no exception. In a study conducted in Jimma Hospital, South West Ethiopia, from March to July 2000, a total of 59 *Salmonella* strains were isolated from 384 pediatric outpatients with diarrhoeal illness (Mache, 2002). This increased isolation rate of *Salmonella* from diarrhoeal pediatric outpatients may indicate poor sanitary conditions. Further studies indicated that the local practice of sanitation was far from satisfactory and that the personal hygiene status of parents responsible for food preparation and child rearing was poor (Beyene *et al*., 2008).

Studies from Ethiopia on HIV co-infection include a study conducted between February and July 2001 in Jimma Hospital. The study identified potential bacterial pathogens in the stool of HIV positive and HIV negative patients. However, of 372 patients (192 HIV+ and 180 HIV-) including 99 HIV positive patients with diarrhoea, a total of 8 *Salmonella* strains were isolated (Beyene *et al*., 2008).

A study was conducted on 400 chicken table eggs from Kombolcha poultry multiplication and breeding farm and market at Kombolcha, Ethiopia. From the total of

400 eggs examined for *Salmonella*, 46 (11.5%) were positive, from which 25 (6.3%) and 27 (6.8%) were found from egg shell and egg content, respectively (Minte *et al*., 2011).

A study was conducted on seroprevalence of pullorum disease in and around Mekelle Tigray region. In this study a total of 770 chicken sera were examined using slide agglutination test with a principle of detecting antibody following infection of the poultries with *Salmonella* Pullorum in the poultry multiplication centre of Mekelle and backyard chickens of the surrounding areas and the prevalence in the local and exotic breeds was 39.3% and 29.2% respectively. Similarly the seroprevalence in age groups less than 6 month, 6-10month and greater than 10 months was also recorded with the prevalence of 5.1%, 35.1% and 34.6% respectively (Netsanet *et al*., 2012).

3. MATERIAL AND MEATHODS

3.1. Study Area

The study was conducted from November 2012 to April 2013 on egg samples obtained from poultry farm at Alage, Ziway and Shashemene and the laboratory work done at Alage Agricultural Technical and Vocational Education Training (ATVET) College of the ministry of agriculture of the Federal Democratic Republic of Ethiopia. The college is located about 217 km southwest of the capital of Ethiopia, Addis Ababa and 32 km west of Bulbula town. The total area of the college covers 4200 hectares and it is situated at longitude of about $38^0$30' east and latitude of $07^0$30' north. The college lies at an altitude of 1600 meters above sea level in the agro-ecologically dry plateau of the south western part of the Ethiopian central rift valley. The area has three distinct seasons, namely main rain, short rain and dry seasons. Based on ten years weather data, the mean annual rainfall of the area is 800 mm with mean minimum and temperature range of 11 and 29 ^{0}C, respectively.

Sampling site

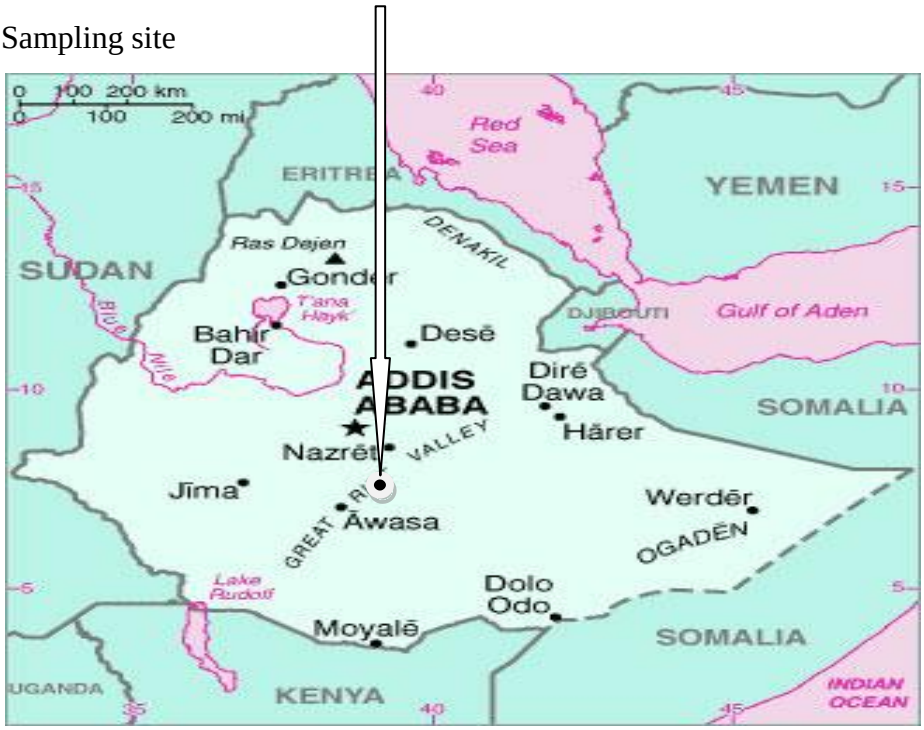

Figure 1. Sample taking area

3.2. Study Sample

The study was conducted on egg from apparently healthy exotic chicken from Alage, Ziway and Shashemene.

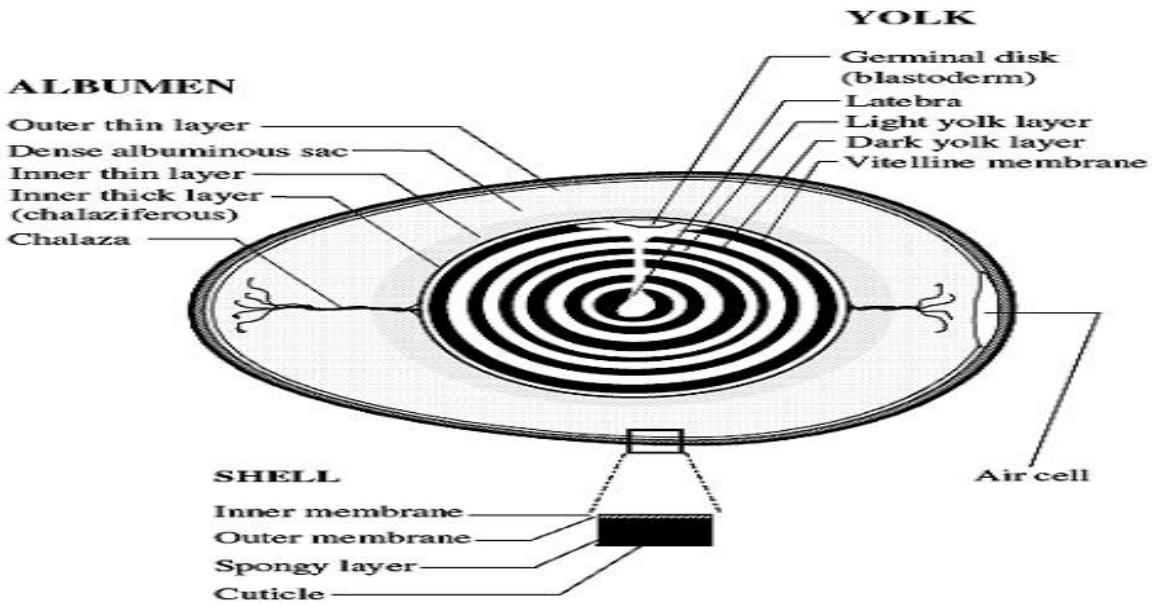

Figure 2: Schematic representation of the parts of the egg (Romo, 2004).

3.3. Study Design

A cross sectional survey was conducted to determine the prevalence and antibiotic susceptibility pattern of *Salmonella* from exotic chicken egg, collected from December 2013 to April 2014. A systematic random sampling of eggs from egg boxes (every 3rd egg from each box) was applied.

Egg sample in sterile polyethylene plastic bags were transported from the farms to Alage Agricultural TVET College, Microbiology Laboratory for examination within 24 hours.

3.4. Sample Size Determination

The number of study eggs was determined based on the expected prevalence of *Salmonella* and the desired absolute precision stated on Thrustfield (2005).

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

Where:- n=required sample size

P_{exp}=Expected prevalence

d= desired absolute precision

Based on a previous study done in Kombolcha poultry multiplication and breeding, the prevalence of *Salmonella* was 15% (Minte *et al.,* 2011). Using these expected prevalence, 95% confidence interval and 5% absolute precision; the number of sampled egg was estimated to be 196.

3.5. Sample Collection and Transportation

Eggs collected directly from the farm using sterile glove at each sample eggs and collected in sterile plastic polyethylene bags, transported to laboratory and examined within 24 hours.

3.6. Laboratory Work

3.6.1. Bacteriological sample processing

The sterile plastic bags containing selected eggs were opened with scissors and the samples processed immediately. Swab technique was used to sample the shell surface of the intact eggs. Sterile cotton swabs dipped in sterile buffered peptone water (BPW) were used to swab the entire surface area of the eggshell. The swabs were directly inoculated into 10 ml BPW in screw capped bottles as described by Suresh (2006).

The same eggs from which shell sample was collected were used for interior (egg content) sampling. The eggs surface were sterilized by immersing in 70% alcohol for 2 min, air dried in a sterile chamber for 10 minute and then cracked with a sterile knife. Each egg's content was collected in sterile universal bottles and homogenize thoroughly by inverting about 25 times as described by ISO6579(2002).

3.6.2. Isolation and identification of Salmonella

Culture method

Microbiological samples for the isolation and identification of this bacteria was processed as described in ISO 6579 (2002);Quinn (2004); OIE (2010).

Non selective pre-enrichment

10 ml BPW was put in 15 ml tubes and swabs immersed in the tubes containing BPW and then the tip of the swab pressed against the wall of the tube to remove excess liquid.

The tip of the swab contacted the surface of the egg and the entire surface swabbed and a second complete sweep at right angles to the first sweep done then the swab returned to the tube with 10 ml BPW in screw capped bottles and incubated at 37°C for 24 hrs. Each egg's content was mixed thoroughly and 25 ml of egg content was inoculated into 225 ml of BPW and incubated at 37°C for 24 hours as described by Harsha *et al*. (2011).

Selective enrichment

The pre-enrichment broth after incubation mixed and 0.1 ml of the broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium (RV broth). The inoculated RV broth then incubated at 41.5 °C for 48 hours as described by Harsha *et al*. (2011).

Plating out and Identification

Plating out and identification done as described in ISO 6579 (2002);Quinn (2004); OIE (2010).

15 ml of XLD poured on one dish for culture on RV then a loopfuls of the cultures streaked on the media. The dishes were inverted so that the bottom was uppermost and incubated at 37^{o}C for 48 hours. After proper incubation, the plates were examined for the presence of suspected *Salmonella* colonies which XLD agar was pink with a darker pink center whereas lactose positive *Salmonella* was yellow with or without blackening.

15 ml BGA was poured on one dish for culture on RV then the plates pre-dried in drying cabinets and loopfuls of the cultures streaked on the media. The dishes were inverted so that the bottom was uppermost and incubated at 37^{o}C for 48 hours. Typical colonies of *Salmonella* on BGA were pink and caused the colour of medium to change to red.

After the detection, the colonies were transferred in to nutrient agar and incubated at 37 ^{o}C for 24 hours for further biochemical test and to preserve the colonies. The plate were divided in to five for each colony to be seeded (four typical and one atypical); When there were fewer than five typical or suspect atypical colonies, all the typical or suspect colonies were taken.

Biochemical tests

Biochemical tests were done as described in ISO 6579 (2002); Quinn (2004); OIE (2010).

Triple sugar iron agar test

5 ml of the medium was dispensed in to the tube with 10 ml capacity and a slant was made by sloping the tubes then colonies were inoculated by stabbing using a loaded straight wire down the centre of the agar and streaked the surface of the slant then incubated at 37 ^{o}C for 24 hours.

Urea broth test

5ml of the media was dispensed in to a test tube and inoculated it with loopfull of the inoculum; after that incubated at 37^{o}C for 24 hours.

Lysine decarboxylation test

A loop full of inoculum was added into 5 ml of the media in a tube, covered the surface with paraffin oil and incubated at 37^{o}C for 24 hours.

Indole test

5 ml of the media was dispensed in to the tube, a loop full of the inoculums added and incubated at 37^{o}C for 24 hours then tested with 0.3 ml Kovacs reagent.

3.6.3. Antimicrobial susceptibility pattern

The antimicrobial susceptibility test was performed following the standard agar disk diffusion method according to CSLI (2008) using commercial antimicrobial disks. The antimicrobials, their symbols and inhibition zone size interpretations are listed in Table 1.

Preparation of the inoculums

The top of at least 5 well isolated colonies touched with a loop and transferred the growth to the tube of saline water. The inoculum emulsified on the inside of the tube to avoid clumping of the cells. To ensure the selection of sufficient bacterial numbers and minimize the risk of selecting bacteria that have lost their resistance, cells picked from more than one colony then the inoculum adjusted to a 0.5 McFarland standard. The turbidity compared with 0.5 McFarland standard using a paper with black lines. The turbidity of inoculum adjusted to match that standard.

Inoculation of Mueller-Hinton plate

Within 15 minutes of preparing the adjusted inoculum, a sterile cotton swab was dipped into the inoculums, the swab rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab then the swab streaked over the entire surface of the Mueller-Hinton agar plate by rotating the plate approximately 60^{o} then repeat streaking motion repeatedly three times. Any excess moisture on the agar surface allowed tobe absorbed prior to applying the antimicrobial disks.

Dispensing antimicrobial disks

The disks were dispensed to the agar surface with a sterile forceps (by flaming with alcohol). After application, the top of the disk touched with forceps to ensure that the disk has made complete contact with the agar surfaceand incubated for 24 hours at 37^{0}C.

Table 1: Antimicrobials used, their symbols and inhibition zone size interpretation for Gram-negative enteric bacteria.

Antimicrobial used [a]	Symbols	Diameter of zone of inhibition in milli meter			
		Resistant ≤	Intermediate	Moderately Susceptible	Susceptible ≥
Amoxycillin (30 µg)	AMC	13	-	14-17	18
Ampicillin (10 µg)	AMP	13	-	14-16	17
Ceftriaxon (30 µg)	CRO	13	14-20	-	21
Chloramphenicol (30 µg)	C	12	13-17	-	18
Ciprofloxacin (5 µg)	CIP	15	-	16-20	21
Gentamycin (10µg)	CN	12	13-14	-	15
Kanamycin (30 µg)	K	13	14-17	-	18
Nalidix acid (30 µg)	NA	13	14-18	-	19
Streptomycine (25 µg)	S	11	12-14	-	15
Sulphamethoxin trimethoprim (25 µg)	SXT	10	-	11-15	16
Tetracycline (30 µg)	TE	14	15-18	-	19

[a] = all are Oxoid products England.

3.7. Data Collection, Management and Analysis

Data describing the presence of *Salmonella* in egg shell and egg content samples was classified, filtered and coded using Microsoft Excel® 2007. The data was then be exported to SPSS windows version 18.0 (SPSS INC. Chicago, IL) for appropriate statistical analysis. The prevalence of *Salmonella* from all samples was determined by using descriptive statistics. Chi square (χ^2) was used and effects reported as statistically significant if p-value is less than 0.05 using 95% confidence intervals.

4. RESULTS

The present study was conducted on 196 non cracked eggs of apparently healthy exotic chickens form Ziway, Alage and Shashemene at Microbiology Laboratory of Alage, Ethiopia from November 2013 to April 2014. Bacteriological examination was conducted on shell and content of each egg sample.

4.1. Prevalence of *Salmonella*

Out of the total 196 exotic chicken table eggs (totally 392 samples)were examined for bacteriological status of *Salmonella*, an overall 13.3% prevalence of *Salmonella* was found. From this, 7.7%and 5.6%were from egg shell and in egg content, respectively. The total prevalence of *Salmonella* varied among the farming system. The prevalence of *Salmonella* in egg content from semi-intensive farm (10%) was significantly higher than the prevalence of *Salmonella* in egg content from intensive farm (3.4%).The prevalence of *Salmonella* in egg shell from semi intensive farm (11.5%) was also significantly higher than the prevalence of *Salmonella* in egg shell of intensive farm (5.7%) as described in table 3.The difference in prevalence observed between egg shell (7.7%) and egg content (5.6%) was statistically significant as shown in table 4.

Table 2: The prevalence of *Salmonella* by sample types and farming type.

	Sample type			
Farming type	Egg content	Egg shell	Egg content and shell (both)	Total
Intensive (n=262)	9(3.4%)	15(5.7%)	3(1.1%)	30(11.5%)
Semi-intensive (n=130)	13(10%)	15(11.5%)	2(1.5%)	22(16.9%)
Total	22(5.6%)	30(7.7%)	5(1.3%)	52(13.3%)

Table 3: The prevalence of *Salmonella* between farms on egg shell and content.

	Semi intensive	Intensive	p-value
Egg shell	15(11.5%)	15(5.7%)	0.021
Egg content	13(10%)	9(3.4%)	0.006
Total	28(21.5%)	24(9.1%)	

Table 4: The prevalence of *Salmonella* in egg shell and egg content.

	No. Of positive	Prevalence (%)	p-value
Egg shell	30	7.7%	0.000
Egg content	22	5.6%	
Total	52	13.3%	

4.2. Antimicrobial Susceptibility of the Isolates

All the 52*Salmonella* isolates were subjected to antimicrobial susceptibility test, using eleven different antimicrobials. Of these only one isolate was resistant to Ciprofloxacin and Ceftriaxon. Ampicillin is highly resisted (55.8%) followed by tetracycline (36.5%), Naldixic acid (30.8%) and Sulphamethoxin-trimethoprim (30.8%). All the results are shown in table 5. Multi drug resistant isolates were found to be 34.6% which is resistant for more than three drugs.

Table 5. Antimicrobial susceptibility pattern of *Salmonella* isolates

	No. (%) susceptible	No. (%) of intermediate	No. (%) of moderately susceptible	No. (%) of resistant
Amoxicillin	35 (67.3%)	-	7	10(19.2%)
Ampicillin	15(28.8%)	-	8(15.4%)	29(55.8%)
Ceftriaxon	51(98.1%)	-	-	1(1.9%)
Chloramphenicol	28(53.8%)	11(21.2%)	-	13(25%)
Ciprofloxacin	50(96.2%)	-	1(1.9%)	1(1.9%)
Gentamycin	46(88.5%)	2(3.8%)	-	4(7.7%)
Kanamycin	22(42.3%)	17(32.7%)	-	13(25%)
Nalidix acid	32(61.5%)	4(7.7%)	-	16(30.8%)
Streptomycin	37(71.2%)	5(9.6%)	-	10(19.2%)
Sulphamethoxin-trimethoprim	32(61.5%)	-	4(7.7%)	16(30.8%)
Tetracycline	18(34.6%)	15(28.8%)	-	19(36.5%)

5. DISCUSSION

Feed contaminated with *Salmonella* has been the most common original source of introduction of new strains of *Salmonella* into livestock production networks, from where it is further distributed by movement of carrier animals and other routes. In many situations international or national trade in livestock or other animals may be the major threat. Feed also may contain less pathogenic 'environmental' serovars that may not be a cause of disease or cycles of infection in animals. As feed contamination may occasionally be caused by *Salmonella* serovars of relevance to public health, feedstuffs should be investigated for the presence of *Salmonella*. As feed is milled with ingredients of mixed global origin a wide range of exotic *Salmonella*, including some serovars that are normally associated with reptiles, may be found in feed. Once established on a holding or integration, spread between animals, environmental contamination and farm pests becomes more important in perpetuating and disseminating the infection (Anon, 2008).

In the current study, *Salmonella* was present in samples such as egg shell swab and egg content. As a result, from the total of 382 samples (196 egg shell and 196 egg content) examined for the status of *Salmonella*, 52samples (13.3%) were positive, from this egg shell was 30 (7.7%) and egg content was 22 (5.6%)positive.

In these findings egg shell positive for *Salmonella* is a little higher than Minte *et al*. (2011) who reported *Salmonella* from egg shells (exterior) was 25 the difference might be due to environmental contamination in the farm during collection as studies have found a correlation between the number of positive environmental samples and the proportion of eggs positive in the flocks (Chemaly *et al*., 2009; Renu *et al*., 2011)and in egg contents a little lesser than Minte *et al*. (2011) who reported egg content (interior) to be 27 (6.8%), that might be due to increased awareness of implementing vaccination.

Of total prevalence in this study is higher than the prevalence of different countries in 2000- 2002 as explained by Ashraf (2008), which is Austria 1.1%, Greece 3.8%, Italy 3.1%, Spain 8.1% and 0% was also reported in France, Germany, Portugal, and USA, in other studies of the same year found 0.5% in UK, 0.19% in Australia, 0.53% in Germany, 0.11% in Italy and 0.03% in Netherlands and 3.3% in England. A 2006/2007 survey of non-UK produced eggs on retail sale in parts of England showed a prevalence of 3.3%. The prevalence difference in this study might be due to contamination in the farm, poor management and the differences between farming system (semi-intensive farming system was included in this study) and environmental factors.

In this study, egg content finding is higher than commercial layer reported by Harsha *et al*., (2011) layer hen which indicated that prevalence of *Salmonella* was recorded 1.8% in egg contents in South India. The difference may be due to proper vaccination program and management implmentation.

The egg shell prevalence in this study (7.7%) which is in agreement with 7.8% finding in Plateau State, Nigeria by Agada *et al*., (2013) and 7% in UK (Adil *et al*., 2012) and it is a little higher than 6.3% report by Minte *et al*., (2011) and have high difference with 0% in Argentina (Favier *et al*., 2001), 0.03% in Saudi Arabia (Alnakhli *et al*., 2000), the difference may be due to environmental contamination, lack of cleaning and disinfecting of materials in the farms.

In this study there is a significant difference between farming system which is the same with a report by Netsanet *et al*., (2012).The low prevalence in the intensive might be due to the reason that in the commercial poultry farms applied routine vaccination programme and have good ventilation, proper spacing of poultry houses and again there is no mixing of breeds (species). But in semi-intensive farming system associated with a limited knowledge such activities may not be performed.

There is a disturbing general trend in *Salmonella* serovars being resistant to commonly used antimicrobials. Antimicrobial resistance among *Salmonella* isolates is increasing worldwide and is likely due to the widespread use of antimicrobial agents for the empiric treatment of febrile syndromes (Bukitwetan *et al*., 2007).

Due to the use of antibiotics for the promotion of growth and prevention of disease in food animals, there is an increase of human salmonellosis cases caused by foodborne MDR *Salmonella* nowadays (Yang *et al*., 2010).

High rates of resistance to Ampicillin, Chloramphenicol, Tetracycline and Trimethoprim-sulfamethoxazole have been reported from many areas of the world (Su *et al*., 2004) which is in agreement with this study being Ampicillin is highly resisted followed by Tetracycline, Nalidix acid and Sulphamethoxin-trimethoprim (equally resisted).

In the study of antibiotic susceptibility test, the following order (highest to lowest) were found- Ampicillin, Tetracycline, Sulphamethoxin-trimethoprim, Nalidix acid, Kanamycin Chloramphenicol, Amoxicillin, Streptomycin, Gentamycin, Ceftriaxon, Ciprofloxacin. The highest level of susceptibility was found in ciprofloxacin which is in agreement with the description of Harsha *et al*., (2011) who put it as ciprofloxacin is a fluoroquinolone antibiotic that is increasingly and successfully used for the treatment of septicemia in humans and Ciprofloxacin resistance in human and veterinary *Salmonella* isolates has occasionally been found.100% of Salmonella isolates were susceptible to Ciprofloxacin (Abdullahi *et al*., 2013) but in this study 96.2% were susceptible and the difference might be due to an increased usage of the drug repeatedly through time.

Study in Neberska by Peter (2006) during 2004, 2005 and 2006 indicated Ceftriaxon resisted 1.7%, 1.8% and 3.2% respectively which is in agreement with this study and

Streptomycin were resisted 19.2%, 24.4% and 21.9% in 2004, 2005 and 2006 respectively, the 2000 report is the same to the present study which is equal.

Ampicillin and Tetracycline are most resisted antibiotic that might be due to it has been used to treat day-old chickens, for humans without prescription to control infection by *Salmonella*, *Escherchia coli* and other diseases, which might have resulted in the emergence of Tetracycline resistant *Salmonella* in the layer and broiler flocks. A similar small scale survey of *Salmonella* from food and humans conducted in Addis Ababa, Ethiopia, during 2003–2004 (Zewdu and Poppe 2009)found 32.7 % of 98 isolates were resistant to one or more of 24antimicrobials tested, with resistance being most common to Streptomycin (75 % which is highly different with this study result which is 19.2%) that might be due to the rate of using this drug inappropriately and the access to this drug was higher in Addis Ababa, followed by ampicillin (59.4 %) which is almost similar with this study reporting 55.8% resistance).

Multi drug resistance rate of 36.6% (18 isolates) was found which is in agreement with the result of 36.4% in Nebraska in 2006 reported by Peter (2006).

6. CONCLUSSIONS AND RECOMMENDATIOS

Salmonella species was found in the shell and contents of eggs of different farms. Prevalence of *Salmonella* was significantly higher in egg shell than egg content which could be the result of contamination due to poor hygiene, handling and absence of cleaning. The total prevalence was high that indicates *Salmonella* contamination in poultry farm is a big concern in farms. Depending on the farming system (intensive and semi-intensive), the prevalence of *Salmonella* in semi-intensive was significantly higher than intensive farming that could be relatively hygienic handling of eggs, cleaning and disinfecting were implemented better in intensive farm. In the study of antibiotic susceptibility pattern of eleven drugs, Ceftriaxon and Ciprofloxacin shows good inhibition on the bacteria. In the other hand most of the drugs shows high rate of resistance especially Ampicillin and Tetracycline. The high rate of resistance and multi drug resistance could be due to drug misuse, drug overuse and inappropriate prescribing practices.

Based on these conclusions the following recommendations are forwarded: -

- ✓ Strict prevention and control measures to reduce the public health risks arising from *Salmonella* should be done by reducing the prevalence hygienic practices and good management.

- ✓ Education and awareness for the public, for consumption of sanitary and well cooked food; farm workers, about appropriate sanitary and hygienic method of production and the risk of food borne diseases should be given.

- ✓ Awareness on the use of drugs and the effect of drug resistance should be given and further detailed research should be done.

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8. ANNEX

Annex 1. Flow diagram for isolation/identification of *Salmonella* from egg

Non-selective pre-enrichment

25 g egg content /shell swab in 10% phosphate buffer (37 °C, 24 h.)

Selective enrichment

0.1 ml in 10 ml Rappaport-VassiliadisBroth (41.5°, 24 h.)

Isolation

XLD with an inoculation loop (37°C, 24 h.)

BGA with an inoculation loop (37°C, 24 h.)

Suspect colonies to nutrient agar (37 °C, 24 h.)

Biochemical confirmation (37 °C, 24 h.)

Triple Sugar Iron Agar (TSI)

Urea broth

Lysine decarboxylation (LIA)

Indole test

Annex2. Plating media record sheet

Sample ----------Place----------Enumerator ----------

Date	Sample number	BGA agar		XLD agar		Sample (+/-)	Comments
		number of		number of			
		typical	Atypical	typical	Atypical		

Annex 3. Biochemical tests record sheet

Sample ---------- place----------Enumerator----------

Date	Sample and colony identification	Test colors /gas							Sal. Positive /negative (+/-)	Comments
		TSI butt	TSI slant	TSI gas	TSI H_2S	Urea	Lysine	indole		

Annex 4. Approximate quantities of media and reagents

Media and reagents	Quantity	Gram /liter	Liters	Amount per 25g sample (ml)	grams per sample (g)	grams for 25 samples	grams for 100 samples	Total samples per 500 g or per liter
Buffered Peptone[a]	500	16.1	31.1	225.0	3.6	90.6	362.3	138
RV broth	500	26.75	18.7	10.0	0.3	6.7	26.8	1869
Selenite broth	500	23	21.7	10.0	0.2	5.8	23.0	2174
GN broth	500	39	12.8	10.0	0.4	9.8	39.0	1282
MacConkey agar	500	50	10.0	10.0	0.5	12.5	50.0	1000
BGA agar	500	58	8.6	15.0	0.9	21.8	87.0	575
XLD agar	500	55	9.1	15.0	0.8	20.6	82.5	606
Nutrient agar	500	23	21.7	15.0	0.3	8.6	34.5	1449
Triple sugar Iron agar	500	64.6	7.7	5.0	0.3	8.1	32.3	1548
Urea broth	500	38.7	12.9	2.0	0.1	1.9	7.7	6460
Mueller-Hinton agar	500	38	13.2	15.0	0.6	14.3	57.0	877

Annex 5. Preparation of media and reagents

Buffered peptone water preparation: 16.1gram of the powder dissolved in distilled water and dispensed into suitable flasks and autoclaved at 121 ^{o}C for 20 min.

Rappaport-Vassiliadis broth preparation: 26.75 gram of the powder was dissolved in 1 litter of distilled water and dispensed into suitable flasks and autoclaved at 121 ^{o}C for 20 min.

Xylose lysine desoxycholate (XLD) agar preparation: 55 gram of the powder dissolved in 1 litter of distilled water. Heated under constant stirring until the medium starts to boil. Immediately transfer the solution to a water bath at about 50^{o}C, continued stirring until the medium has reached about 50^{o}C.

Brilliant Green Agar (BGA) preparation: 58 gm per litter of the dehydrated medium dissolved in distilled water by heating to the boiling point for 1 minute and transfer to sterile 1000 ml bottles.

Nutrient agar preparation: 28 gram the dehydrated medium dissolved in the distilled water by heating. Transferred into bottles and autoclaved it at 121^{0}C for 15 minute.15 ml of melted medium poured into each sterile 10 cm petri dish.

Mueller-Hinton Agar was prepared by mixing 38 gram per litter of distilled water. And 25 ml of the media dispensed in a petridish.

Reagent and media preparation continued

Saline solution preparation: Dissolve 8.5 gm of sodium chloride in 1 litter of distilled water. The solution dispensed into tubes so 4 ml is obtained after autoclaving at 121 ^{0}C for 25minute.

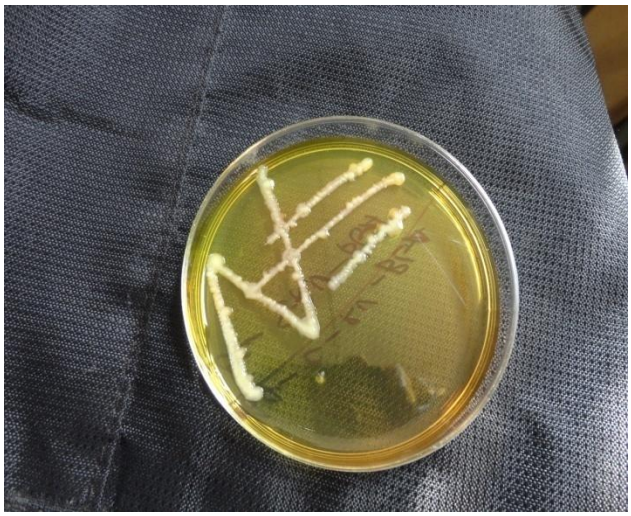
L-Lysine decarboxylation medium preparation: 14 gram of the powder dissolved in distilled water (boiling 2 min may be necessary)and autoclaved at 121^{o}C for 15 minute.

Triple sugar/iron agar (TSI agar) preparation: 64.6 gram of the dehydrated medium dissolved in 1 litter of distilled water by heating and autoclaved at 121^{o}C for 15 minute.

Urea broth preparation: 38.7 gram of the dehydrated base dissolved in distilled water by heating and sterilized in the autoclave at 121^{o}C for 15 minute.

Indole test: 16 gram of the medium dissolved in distilled water at by heating and autoclaved at 121^{o}C for 15 min.

Annex 6. Pictures of *Salmonella* isolates in different media



Salmonella on BGA

Salmonella on XLD

9. CURRICULUM VITAE

A. Biographical Data:

Name	SOLOMON TSEGAYE ADANE
Date of birth	May12, 1981 G.C. (4/10/ 1973 E.C.)
Place of birth	Kombolcha, Ethiopia
Marital status	Married
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Senior Instructor

B. Educational background

Year (E.C.)	School Attended
2011-2014	Masters study in Addis Ababa university, CVMA, Bishoftu, Ethiopia.
2002-2007	DVM study in Addis Ababa University, Faculty of Veterinary Medicine, DebreZeit, Ethiopia
1994-2000	Kombolcha secondary School, Kombolcha

C. Work Experience

November, 2007 till now Working positions	Senior Instructor at Alage Agricultural Technical and Vocational Training Collage.
	From 2009-2012- Department head of animal health at

Alage ATVET college.

D. Research output/Technical paper

Solomon T. and Teshale, S. (2007): Examination of gross pathology of cattle lung in Jimma. An undergraduate thesis paper, Addis Ababa University, Faculty of Veterinary Medicine.

Solomon T., Aklilu, F. and Getachew, T. (2014): The prevalence and antimicrobial susceptibility pattern of *Salmonella* isolates of exotic chicken eggs from Alage and surrounding towns. Ethiopia. An Msc thesis paper, Addis Ababa University, CVMA.

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10. SIGNED DECLARATION SHEET

Statement of author

First, I declare that this thesis is my work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfilment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Name:_____ Signature:_____

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Date of Submission:_____