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ISOLATION AND IDENTIFICATION OF *SALMONELLA* SPECIES FROM SMALLHOLDER BROILER CHICKEN FARMS AND THEIR ANTIBIOGRAMS IN CENTRAL ETHIOPIA: ITS IMPLICATION FOR PUBLIC HEALTH

BY

TESFAYE BELACHEW

JUNE, 2021

BISHOFTU, ETHIOPIA

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

Isolation and Identification of *Salmonella* species from Smallholder Broiler Chicken Farms and Their Antibiograms in Central Ethiopia: Its Implication for Public Health

A Thesis Submitted to Addis Ababa University College of Veterinary Medicine in partial fulfillment of the requirement for the degree of master of Veterinary Medicine in Veterinary Epidemiology

By:

Tesfaye Belachew

MSc Program in veterinary Epidemiology

Advisors: Teshale Sori (DVM, MSc, PhD, Associate Professor)

JUNE, 2021

BISHOFTU, ETHIOPIA

Addis Ababa University

College Of Veterinary Medicine and Agriculture

Department of Clinical Studies

As MSc research advisors, we here certify that we have read and evaluated the Thesis prepared under my guidance by Tesfaye Belachew entitled**: "Isolation and Identification of *Salmonella* species from Smallholder Broiler Chicken Farms and Their Antibiograms in Central Ethiopia: Its Implication for Public Health**"; we recommend that it can be submitted as fulfilling the MSc thesis requirement.

Submitted by:

Tesfaye Belachew _____ _____

Name of Student Signature Date

Approved for submittal to thesis assessment committee:

1. Teshale Sori (DVM, MSc, PhD, Associate Professor)

Major Advisor _____ _____

Signature Date

Dr. Haileleul Negussie (DVM, MSc, PhD, Associate Professor)

Department chairperson _____ _____

Signature Date

APPROVAL

Addis Ababa University
College Of Veterinary Medicine and Agriculture
Department of Clinical Studies

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: Tesfaye Belachew entitled "**Isolation and Identification of *Salmonella* species from Smallholder Broiler Chicken Farms and their Antibiograms in Central Ethiopia: Its Implication for Public Health**" and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Veterinary Epidemiology.

Megersa Bedasa (DVM, MVSc, Assist.Prof.)
Chairman _____ _____
Signature Date

Kasahu Amsalu (Dr.)
External Examiner _____ _____
Signature Date

Zerihun Assefa (DVM, MVSc, Assist.Prof.)
Internal Examiner _____ _____
Signature Date

Haileleul Negussie (DVM, MSc, PhD, Associate Professor)
Department chairperson _____ _____
Signature Date

Dr. Teshale Sori (DVM, MSc, PhD, Associate Professor)
Major Advisor _____ _____
Signature Date

STATEMENT OF AUTHOR

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Name Tesfaye Belachew Signature _____

College of Veterinary Medicine, Bishoftu, Ethiopia

Date of Submission: _____

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LIST OF ABBREVIATIONS

BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
CSA	Central Statistics Agency
EMB	Eosin Methylene Blue
GBD	Global Burden of Disease
GIT	Gastro Intestinal Tract
ISO	International Standardization Organization
LPS	Lipopolysaccharide
NSAID	Non-Steroidal Anti-Inflammatory
NTS	None Typhoidal *Salmonella*
PMDC	Poultry Multiplication and Distribution Centers
RVS	Rappaport-Vassiliadis soy pepton
SPI	*Salmonella* Pathogenicity Islands
SSA	*Salmonella* Shigella Agar
TSIA	Triple Sugar Iron Agar
UV	Ultraviolet
VP	Voges Proskauer
XLD	Xylose Lysine Deoxycholate

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ABSTRACT

Broiler meat is the second most widely consumed meat and it is one of the sources of zoonotic *Salmonella* serotypes. The study was assessed the occurrence of Salmonella isolates in smallholder broilers chickens and the antibiotic susceptibility profiles of Salmonella isolated from the study farms. From November 2020 to May 2021, a cross-sectional study was undertaken on smallholder broilers farms and slaughter house in Bishoftu and Modjo to assess the occurrences of Salmonella species and to detect their antimicrobial resistance pattern. From selected farms selected (two farms from each) a total of 289 samples (189 cloacal swab samples, 52 feed and 48 water samples) were collected from broilers farms whereas 100 cecal contents were collected from slaughtered chicken at Chico-Meat slaughter house in Bishoftu and 26.46 % of the cloacal samples, 21.00 % of the cecal contents, 30.77 % of the feed samples and 25.00 % of the water samples yielded Salmonella. Total of 389 samples were collected analyzed in the laboratory using standard bacteriological techniques and typical Salmonella colonies were further characterized by biochemical test. Antimicrobial susceptibility of the isolates was conducted using standard Kirby-Bauer disk diffusion method. The results of questionnaire survey showed that all farmers and attendants did not have knowledge about the occurrence of Salmonella in broilers. Overall 99 samples (25.45 %) were positive results for *Salmonella*. Of the samples collected from Bishoftu 43 of the 200 (21.50 %) were positive whereas 56 of the 189 (29.63 %) samples collected from Modjo were found positive. The effect of potential risk factors such as age ,breed, source of water ,type of house and farm location on the occurrence of Salmonella was assessed using logistic regression with odd ratio and breed was the only significantly ($p < 0.05$). The results of antimicrobial susceptibility test showed that the *Salmonella* isolates were resistance to Tetracycline (80 isolates, 80.81%), Kanamycin (71 isolates, 71.72%), Chloramphenicol and Amoxicillin (67 isolates, 67.68%) whereas most of the isolates were susceptible to Gentamicin (69 isolates, 69.70%) and Erythromycin (40 isolates, 40.41%). The results of this study showed that *Salmonella* isolates in broiler and their inputs were resistant to most of antimicrobials used in medical and poultry practices. This has important implication for public health.

Keywords: *Antibiotics, Broilers, central Ethiopia, Resistance, Salmonella, Smallholders*

1. INTRODUCTION

Food borne diseases are a particularly series challenge to human health worldwide. Within the past few years, the epidemiology of food borne diseases has been increased as a consequence of changes in the social environment and therefore the ability of pathogens to adapt to new niches. In Ethiopia poultry development endeavors, however, are likely to be challenged by prevailing diseases and feed shortage, among others. Diseases have already been very important constraints of poultry production in Ethiopia and affect both village (extensive) and intensive poultry production. Food-borne pathogens are the causes of diseases and death in developed and developing countries, which is leading to the loss of labor and could have contributed within the economic growth importance (Crump *et al*., 2015). Food related diseases are mostly caused by changes in dietary habits, mass catering, unhealthy food storage conditions, and bad hygiene practices (Peal *et al*., 2020). The risk of the transmission of zoonotic infections are also associated with contaminated feaces, egg and meat, milk and milk products (Alders *et al*., 2018).

Broiler meat is the second most popular meat in the world, accounting for around 36% of total meat production (Conway, 2017). *Salmonella* serotypes are the major bacterial pathogens of poultry worldwide, and most *Salmonella* illness in peoples occurred from the intake of contaminated meat broilers and their products (Carli *et al*., 2001). *Salmonella pullorum* (*S. pullorum*), *Salmonella gallinarum* (*S. gallinarum*) and *Salmonella enteritidis* (*S. enteritidis)* infect the ovaries of hens and transmitted through eggs. Pullorum disease, also known as bacillary white diarrhea, affects young poultry and turkey poults aged 2 to 3 weeks. The mortality rate is high, and the infected birds are anorexic and depressed when they huddled under a heat source. The production environment and the level of management provided at the farm level in developing countries like Ethiopia is not the standard demanded (Ejo *et al*., 2016). *Salmonellosis* is considered to be one of the most foods borne diseases in humans, with globally and are economically important disease of all animals' species (Foley *et al*., 2013). *Salmonella* contamination is a major public health issue around the world, putting a huge financial burden on both developed and developing countries due to the costs of disease control, prevention, and care (Crump *et al*., 2015; Vernooij *et al*., 2012). *Salmonella* spp. are

potentially responsible for various pathogenic processes in humans and animal including poultry (Imen *et al*., 2012). *Salmonella* cause diarrhea, vomiting, fever, abdominal cramps in human and cases, where *Salmonella* enters into the bloodstream, symptoms include high fever, malaise, pain in the thorax and abdomen, chills and anorexia (WHO, 2002). Effective control systems are in controlling product safety, and considerable information is available on how to minimize the risks (FAO, 2013).

Misuse of antimicrobials increases the number of single and multidrug resistant *Salmonella* bacteria strains, were identified by the World Health Organization and health authorities as they were having serious problems in global public health and veterinary sector and is growing as infections caused by resistant bacteria become more difficult and costly to treat. The rising various of single and multiple drug resistant of *Salmonella* strains identified from human cases have linked to the widespread use of antimicrobial drugs in broilers and other food animal production and their products, this constitutes a risk to public health due to the transmission of resistant *Salmonella* strains to humans via infected food and food products. (Bada Alambedji *et al*., 2006). Antimicrobial resistance in *Salmonellosis* can be reason to occur higher morbidity, mortality, and costs treatment. In addition to this, it will require critical Scientific and Public Health efforts to bolster events with social and economic impact (Bada-Alambedji *et al*., 2006).

In Ethiopia there is little information, but there is a suggestion that the camp population has a low level of typhoid carriage. Children in developing countries, including Ethiopia, are particularly susceptible to salmonellosis. During the last few years, there has been gradual increase in commercial small and medium scale, market oriented poultry production. This reflects the efforts of the Government of Ethiopia to facilitate the productive basis of domestic birds within a genetic improvement programed by introducing and distributing exotic breeds, provide improved extension advice, training ,services and to generally increases the capacity of the sector to boost rural productivity (with the implications therein for raising incomes, providing employment and alleviating poverty). These plan have been introduced courtesy of poultry multiplication and distribution centers (PMDC) (Gezahegn and Rich, 2010).

In some parts of Ethiopia studies were conducted and identified the presence of *Salmonella* infection in animals, humans, foodstuffs and environment (Abebe *et al*.,

2020; Woldemariam *et al*., 2005). With the higher increasing of poultry industry and the fast expansion of poultry (broilers) chicken on the world made *Salmonellosis* to rank as one of the most important food born bacterial disease of poultry.

General Objective

Therefore, the general objective of this study was designed to assess the occurrence of *Salmonella* isolates in smallholder broilers chickens' farm in Bishoftu and Modjo towns and the antibiotic susceptibility profiles of Salmonella isolated from the study farms.

Specific Objectives

- To assess the occurrence of salmonella in cloacal swab, cecum, feed and water
- To estimate prevalence and associated risk factors of *Salmonella* isolates
- To determine the antimicrobials susceptibility profile of the salmonella isolate

2. LITERATURE REVIEW

2.1. Classification and Nomenclature

The genus *Salmonella* belongs to the bacterial family of *Enterobacteriaceae* and it was named after the American Veterinary Microbiologist Dr Daniel Elmer Salmon (1850-1914). Salmonella from complex group of bacteria consist of two species; *S.enterica and S.bongori* Subsequently, Kauffman and White classified *Salmonella* into more than 2,500 serotypes (Münch *et al*., 2012). Most of *Salmonella* serotypes are group of *Salmonella enterica* sub species *enterica* (*S. enterica* subsp. *enterica*) as shown in figure 1. Within *S. enterica* subsp. *enterica*, the most common O antigen serogroups are A, B, C1, C2, D and E. Strains in these serogroups cause near to 99% of *Salmonella* diseases in humans and warm-blooded animals (Brenner *et al*., 2000). Serotypes in *S. enterica* subsp. II (*S. enterica* subsp. *salamae*), IIIa (*S.enterica* subsp. *arizonae*), IIIb (*S. enterica* subsp. *diarozonae),* IV (*S. enterica* subsp. *indica*), and V (*S. enterica* subsp. *bongori*) are almost all identified from cold blooded animals, environment and also sometimes from humans. Strains of *Salmonella* are categorized into serovars based on extensive diversity of lipopolysaccharide (LPS) (O) antigens and flagellar protein (H) antigens in accordance with the Kauffmann/White scheme. Except for serotypes *S. Typhi, S. Paratyphi A,* and *S. Paratyphi C,* which are specific to human and whose only reservoir are human being and other all serotypes can be identified as zoonotic or source of zoonotic (Baird-Parker, 1990 ; Acha and Szyfres, 2001).

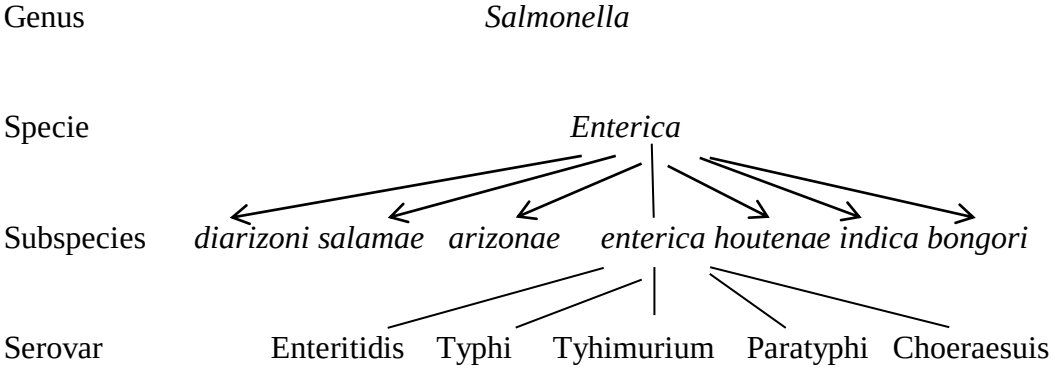

Figure 1: Schematic presentation of the current Nomenclature of *Salmonella*

2.2. Susceptibility of Salmonella

Salmonella are normally heat sensitive and cooking destroys *Salmonella* in poultry meat and heat (for example 57°C for 70 minutes) eliminate *salmonella* that are inside intact eggs and other poultry products(Yadav *et al*., 2016). Heat/steam treatment is commonly practiced to rid poultry feed of *Salmonella*. At lower temperatures this is commonly referred to as 'pelleting' while at higher temperatures it is known as 'conditioning'. Irradiation will remove *Salmonella* from poultry products but in most countries this practice is not liked by consumers. Disinfectants kill *Salmonella* but they do not always totally rid a poultry house of *Salmonella*. Various chemicals have been used to rid poultry feeds of *Salmonella*. *S. enteritis* has been known to survive in poultry litter and feed for more than two years but in litter its ability to survive depends upon litter pH and water activity (Ramesh *et al*., 2002).

2.3. Virulence factors

The pathogenicity of paratyphoid *Salmonella* can be influenced by two toxin endotoxin and enterotoxin types. The endotoxin is linked to the lipid A portion of the cell wall lipopolysaccharide of the *Salmonella* bacterium. This endotoxin, if it gets into the poultry bloodstream when *Salmonella* organisms are lysed (destroyed), is able to produce fever (Bertelloni *et al*., 2017). The lipopolysaccharide inside *Salmonella*'s cell wall also helps the *Salmonella* bacteria to resist attack and digestion by phagocytes in the poultry immune system. If *Salmonella* cannot synthesis complete lipopolysaccharide the ability of certain serotypes of *Salmonella*, such as *S. typhimurium*, to colonize the caeca and/or invade the spleen is impaired. Toxins made from protein, known as enterotoxins, induce a secretory response by epithelial cells (cells lining the digestive tract and other systems) that results in a fluid accumulation in the intestine that can contribute to digestive upset or scouring (Rychlik *et al*., 2009).

2.4. Adherence and Invasiveness

These two properties relate to paratyphoid *Salmonella*'s ability to adhere to and then invade epithelial cells. These are the first two key levels in this bacterium's capacity to cause disease. Serotypes of *Salmonella* with a reduced ability to function in this way also

tend to be among the weaker strains when it comes to producing disease. The fimbriae and flagella on the surface of the *Salmonella* bacterium play a role in this process and strains of *S. enteritidis* lacking such structures are also less able to adhere to epithelial cells and are poor colonizers of the caeca (Andino and Hanning, 2015). Fimbriae and flagella are essential for *S. enteritidis* to be capable to colonize the poultry digestive tract. *Salmonella's* potential to penetrate the lining of the digestive tract following adherence and the organism's adhesion and penetration properties are personally regulated. Mutant strains of *S. enteritidis* that are unable to colonize the bird's digestive tract are still virulent after intra-peritoneal administration (Olsen *et al*., 2013). Adherence and invasiveness properties of *Salmonella* organisms can be influenced by way of laboratory cultural strategies and so one has to be careful in decoding the residences of *Salmonella* strains that have been multiplied up by means of cultural techniques in the laboratory. Changes to the environment that a *Salmonella* organism finds itself in, within the poultry digestive tract, can cause changes in the expression of virulence related genes (Kim *et al*., 2013).

2.5. Economic Importance

The importance of S*almonella* infections in economic terms cannot be overlooked and cause infection in broilers with significant losses and this is a real cost to the producers. When human food poisoning occurs there are losses to the victim in terms of loss of income and to society in terms of lost productivity. The presence of *Salmonella* in broilers or broilers products hinders international trade (Antunes *et al*., 2016). *Salmonella* in a foodstuff can influence its acceptability by consumers and this can significantly adversely affect specific businesses. Nowadays, *Salmonella* and its control and monitoring bring an important value to most broilers chicken farmers. Finally, in many countries certain types of flocks are slaughtered or they or their products are directed down less financially rewarding channels. *Salmonellosis* is a common human being of intestinal primarily due to *Salmonella* in meat and broiler contamination. The value of chicken is estimated at thousands of dollars annually to countries, so draining cash which could have been utilized to development of the country (WHO, 2009). *Salmonella* species are a substantial source of economic loss in broiler chicken farms due to the expense of clinical disease, which includes fatalities, diagnosis and treatment of clinical cases, diagnosis laboratory expenses, cleaning and disinfection expenses, and

control and preventive expenses. The loss incurred by the livestock producers includes feed efficiency, and reduced weight gains or deaths because of *Salmonellosis* (Woldemariam *et al*., 2005).

Human foodborne *Salmonellosis* constitutes a major health problem in many countries and the costs associated with *Salmonellosis* could be considerable. Financial expenditures are related not only with human illness inquiry, treatment and prevention but also with the industrial system chain. Poultry products have always high ranked in the incidence of *Salmonellosis* in most of developing countries including India, Egypt, Brazil and Zimbabwe (Henson, 2003) and is the most seriously perceived food risks in Broilers chicken meat, even in the developed countries as well.

2.6. Risk Factors for Salmonella Infection

Most animals are more likely to develop disease if their normal flora is interrupted (for example stress, antibiotics). Animals are more vulnerable to exogenous exposure or the activation of silent infections under these conditions. *Salmonellosis* affects young animals more than it does older animals. Factor that predisposes animals to clinical salmonellosis include inadequate hygiene, overcrowding, adverse weather, hospitalizing stress and surgery, parturition, parasitism, transportation and competitor viral infection (Rukambile *et al.*, 2019). During their lives, most of animals have inapparent infections. This particularly applies for rations containing *Salmonella* and the subclinical form of *Salmonella* in swine and poultry. An animal may be latent and contain a pathogen in the lymphatic nodes or a transporter and remove the agent in its fecal wastes occasionally or continuously (Demirbilek, 2017). The risk of shedding *Salmonella* seems to vary according to production system, housing type, general level of sanitation as well as management type and animal age. Large herd size is major risk factors for *Salmonellosis*. *Salmonella* physiology has made it harder in controlling environmental contamination and transmission of the bacteria. *Salmonella* are most serious bacteria with a many of potential vehicles, vectors and reservoirs within a poultry flock. *Salmonella* are facultative anaerobes and can grow well under both aerobic and anaerobic conditions. The optimum temperature to support growth is 37.5°C, but *Salmonella* can grow over a range of 5 to 42°C and can grow within a pH range of 4.0 to 9.0, with an optimum pH 7.0. Cockroaches and lesser mealworms could carry *Salmonella* internally and externally

and spread throughout the poultry house. *Salmonella* vectors in feed have also been identified in non-biting flies, fleas, ticks, and bread beetles. Mice were also one of the most important vectors for *S. Enteritidis* in laying poultry (Myint, 2004). Wild birds and lizards are important reservoirs of *Salmonella* and can transmit *Salmonella* infection in different ways to broilers chicken flocks.

The sources of bacterial contamination from feed, poultry products, and even human *Salmonella*, are widely believed in insect and animal vectors. The agricultural environment can also be a reservoir. The physical and feeding conditions are important reservoirs in which *Salmonella* is able to last for a long time. *Salmonella* Infections can spread among broilers chickens' flocks through direct contact with infected broilers chicken and contaminated environment. *Salmonella* infected food or water are most important source of *Salmonella* infections (Craven *et al*., 2000). The standard dose for human to be infested with *Salmonellosis* has been reported to range from 1-109 cfu associated with type of food ingested, immune status of the host and virulence factor of the Salmonella strains. When ingested with liquid food (e.g., milk and others), foods that neutralize gastric acid (e.g., cheese) and then the pathogen carries a high virulence gene, the infectious dose decreases (Arun, 2008).

The increased movement of human, animals and food products into and out of borders, urban growth in developing countries, an increase in the number of immune-compromised people, changes in the food management and intake and the launch of various or antibiotic resistant infectious agents are all contribute to an increase in food safety risk (Unnevehr, 2003).

2.7. Salmonella Infections in Poultry

Salmonella can be found in a wide range of domestic and wild animal hosts (Campos *et al*., 2018). The infection may or may not be clinically seen in both domestic and wild animals. In the form of the sub-clinical case, the animal could have a latent infection and carry infectious *Salmonella* in its lymph nodes or it might be a carrier and intermittent or persistently eliminate the agent in its fecal content. In domestic animals, there are number of common scientific enteritis because of species-adapted serotypes, like *S. pullorum or S. abortusequi.* Clinically apparent or in apparent diseases are caused by

serotypes with different hosts (Marin *et al*., 2018). Two serotypes, *S. pullorum* and *S. gallinarum*, are mostly infect poultry flocks. They are not more infectious for human beings, although cases of *Salmonellosis* caused by these serotypes have been isolated in children. Numbers of other serotypes are repeatedly isolated from domestic birds; as result, these domestic birds are considered as the one of the main reservoirs of Pullorum infections caused by serotype *S. pullorum*, and chicken typhoid. *S. gallinarum*, produce the highest economic losses on broilers chicken farms if not properly managed. Both illnesses are spread globally and give rise to highly morbid and fatal outbreaks. During the first 2 weeks of birth the pullorum illness occurs and causes high levels, with a vertical and horizontal transmission and infected eggs contaminating incubators and hatchers were laid by the carrier poultries (Shivaprasad and Barrow, 2008).

Typhoid occurred commonly in human, poultry and is transmitted through the fecal content of carrier birds. On an affected broilers chicken farm, getting better birds and curiously healthy birds are reservoirs of infection. *Salmonella* un-adapted to fowl also infect them frequently. Almost all the serotypes that attack humans infect poultry as well. Some of these serotypes are isolated from healthy poultry. The infections in adult poultry were shown asymptomatic, but during the first two to three weeks of life, its clinical signs the same to Pullorum disease (loss of appetite, nervous symptoms, and blockage of the cloaca by diarrheal fecal matter). The highest mortality presented during the first two weeks of life and most deaths occur between six to ten days after hatching(Parin *et al.*, 2018).

2.8. Salmonella Infection in Humans

In humans *Salmonella* infections can range from self-limited non-typhoidal *Salmonella* (NTS) gastroenteritis to typhoidal fever, which may lead to fatal perforation in the bowel (OIE, 2000). Non-typhoidal *Salmonella* infection is the most causes of food poisoning on the world with an approximated annual incidence of 1.3 billion cases and 3 million losses each year (Forshell and Wierup, 2006).The occurrence of outbreaks of salmonellosis have been reported by different researchers for many years in different parties of the world, but within the past few years the disease has expanded in incidence in most of world countries. The *Salmonella* infections appears to be most occurred in areas of highly intensive animal farming system practiced (OIE, 2000). The incubation period in

human is different but is most probably between 12 to 36 hours. The specific clinical sign is diarrhea but this may followed by nausea and abdominal pain and some cases we can see headache and fever. While the diseases is normally self-limiting and does not require medication sometimes with more invasive *Salmonella* such as *S. Virchow*, bacteremia can occur and the infection rarely causes death in humans (Feasey *et al*., 2012).

The most common cause of *Salmonellosis* is S. Typhimurium or S. Enteritidis. Secondly, *S. enterica* subsp. *typhi* and *S. enterica* subsp. *paratyphi* are the causes of both typhoid fever and paratyphoid fever, respectively. *Salmonella* can multiply both inside the vacuoles of animal and human cells and in the around environment. In developed countries, *Salmonella* is the second most prevalent pathogen isolated from humans with gastroenteritis (Small *et al*., 2006). *S. typhimurium* and *S. enteritidis* are found in the gastro-intestinal tracts of humans and animals including livestock. *Salmonella infection* is self-limiting but can be infecting younger, elderly or otherwise immune compromised people. *Salmonella* invade epithelial cells inside the ileum and proliferate in the lamina propria and profuse, watery diarrhea results. Few isolates of *Salmonella* produce a heat-labile enterotoxin, which initiates diarrhea (Ferede, 2014). Sequelae are post-enteritis reactive arthritis and reiter's syndrome and systemic infection can cause and each single can create carrier status of up to 6 months in duration. The infectious dose varies, from only a few colonies forming unit to less than 105 colonies forming unit (CFU), As a result, pathogen growth in foods does not appear to have been a factor in all cases of foodborne *Salmonellosis*, but it appears to have been in some cases. Foods that have been linked to salmonellosis include chicken, eggs, and meat, as well as milk, chocolate, coconut, and frog legs, and any faecally contaminated food (Small *et al*.,2006). *Salmonella typhi* and *S. enterica* subsp. *paratyphi* can cause the systemic diseases typhoid fever and paratyphoid fever, respectively in peoples. These bacteria occur in human faeces, and are transmitted via human faeces to the environment and to foods and Person to person transmission is common. The disease clinical sign of typhoid and paratyphoid fevers are not the same to those of enteric salmonellosis. *Salmonella* penetrate the intestine wall and inter into the intestinal epithelium, possibly proliferating in macrophages and polymorphs, and then pass into mesenteric lymph nodes, liver or spleen and then septicemia occurs(Dougan *et al.*, 2011). There may be occasionally peritonitis and subsequent death and ulceration of the ileum might lead to multiplication

bacteria in bile and the re-infection of the gallbladder. Any foods that are contaminated by infected human and other elements could be an infection vehicle. Raw milk, shellfish and other meat products are known to have been vehicles for typhoid illness. However, typhoid illness is mostly transmitted to human by contaminated water from human feaces (Hammarlöf *et al*., 2018).

2.9. Epidemiology of Salmonella Infections

The epidemiology of *Salmonella* is complicated due to broad range of hosts and vectors (for effective use of transmission), the potential to continue to survive for a long time in the climate, persist in the population due to efficient fecal shedding from carrier animals and plentiful reservoir hosts therefore, it have mastered virtually all of the attributes necessary to make sure big distribution (Ferrari *et al*., 2019). In 2016, the overall number of chickens was projected to be 59.5 million. The high poultry mortality due to diseases and predators prevalent in scavenging production systems is the key reason for the stagnation of poultry population growth. Approximately 32 million birds died from disease, and close to 30 million birds were lost due to causes other than disease, most of which predate the prevalence rate of *Salmonella* in broilers and laying breeding reproducers in Batna were 12% and 1.6% for *S. typhimurium* and *S. livingstone* respectively. The poultry faeces and litter were the only samples contaminated with these pathogens (CSA, 2007).

Another factor is the small growth of commercial poultry production in terms of both the number of operators and the amount of production *Salmonella* was found in 12 percent of broilers and 1.6 % of laying breeding reproducers in Batna were 12% and 1.6% for *S. typhimurium*. *Salmonella* are common in the area, and can be present in agricultural effluents, human waste, and other feaces-infected materials. Salmonellosis has been found in every world, but it seems to be highly occurred in intensive animal productions system, mostly in poultry and swine production.

Salmonellosis epidemiology is complicated by the fact that there are over 2,500 different serotypes, each with different reservoirs and local incidences. Changes in food consumption(intake), production, and distribution have led to an increasing frequency of multistate outbreaks associated with fresh produced and processed foods (Rounds *et al*.,

2010). The report WHO Global *Salmonella* Survey, during 2000-2002, the most common serotype reported from humans worldwide was *S. enteritidis* and it accounted for 65%, followed by *S. typhimurium* (12%) and S. newport at (4%). From animals' isolates, *S. typhimurium* was the most prevalent isolate reported serotype accounting for (17%) followed by *S. heidelberg* (11%) and *S. enteritidis* (9%). *S. enteritidis, S. typhimurium* and *S. typhi* were highly prevalent among the 15 most common human serotypes in all over the world throughout the three year study period. *Salmonella Agonaagona, S. infantis, S. montevideo, S. saintpaul, S. hadar, S. mbandaka, S. newport, S. thompson, S. heidelberg* and *S. virchow* were also widespread. In Africa in 2002, S. enteritidis and S. typhimurium were each reported from nearly 1/4 of isolates from humans. In 2000, typhoid fever was estimated to cause nearly 21.7 million illnesses and 216,000 losses and paratyphoid fever 5.4 million illnesses (Crump *et al*., 2004).Typhoid and paratyphoid fevers were estimated to account for 12.2 million disability-adjusted life years in the Global Burden of Disease 2010 project (GBD 2010) (Rudd *et al*., 2020). In the report of the International Vaccine Institute, typhoid fever caused 11.9 million diseases and 129,000 deaths in low and middle countries in 2010. In some Asian countries, *Salmonella* serovar paratyphi A has accounted for a growing proportion of enteric fever (Fagbamila *et al*., 2017).

In Ethiopia, there is limited information on the isolation and antimicrobial resistance of *Salmonella* from chicken eggs and meats. There was a scarcity of information about *Salmonella* and poultry farm(Aragaw *et al.*, 2010; Kindu and Addis, 2013) on sources related to poultry, with a focus on *S. gallinarum* and *S. pullorum*. In a study carried out in a rehabilitation center, camp in Korem, a total of 42 (21.1%) of the camp residents had a positive *Enterobacteriaceae* stool culture, but only 2% of them were *Salmonella* species. These were not investigated any further. There is little information in this study, but there is a suggestion that the camp population has a low level of typhoid carriage. Children in developing countries, including Ethiopia, are particularly susceptible to salmonellosis. A total of 59 *Salmonella* strains were isolated from 384 pediatric outpatients with diarrhoea in a study conducted in Jimma Hospital in South West Ethiopia from March to July 2000 (Mache, 2002). At Kombolcha in Ethiopia, a study was performed on 400 chicken table eggs from the Kombolcha poultry multiplication and breeding farm and market. 46 (11.5 percent) of the 27 400 eggs tested for *Salmonella*

were positive, with 25 (6.3 percent) and 27 (6.8%) were contained in the egg shell and egg material, respectively (Assefa *et al*., 2011).

In and around Mekelle, Tigray study was conducted on seroprevalence of pullorum disease on a total of 770 chickens using a slide agglutination test with the prevalence in the local and exotic breeds being 39.3 percent and 29.2 percent, respectively. Seroprevalence was also measured in age ranges of less than 6 months, 6-10 months, and greater than 10 months, with prevalence of 5.1 percent, 35.1 percent, and 34.6 percent, respectively (Berhe *et al*., 2012).

2.10. Salmonella Transmission

Salmonella organisms are passed from animals to humans and from humans to humans through the feaces oral route. *Salmonella* can be shed in the stool of pets for 4 to 6 weeks after infection (Swanson *et al*., 2007). The method by which *Salmonella* infect eggs was proposed as shell penetration and transovarian transmission with three serotypes *S. enteritidis, S. typhimurium* and *S. newport,* which were isolated from chicken ovaries and feaces are a result of contaminated laying hens (Gantois *et al*., 2009). Vertical transmission of *Salmonella* in poultry is transmitted from infected reproductive tissues to eggs prior to shell formation and different *Salmonella* serotypes able to infect egg contents by migration through the egg shell and membranes. Moisture in the egg shells, storage at room temperature and shell damage all help to facilitate this route. Horizontal transmission can infect by direct bird to-bird contact, consuming of contaminated feaces or litter, contaminated water, infected personnel, farm and personal equipment, and a variety of other sources. Backyard hens can also contaminated through contact with wild animals, domestic mammals and commercial poultry that are carriers of *Salmonella* and consequently may play a role in the *Salmonella* bacteria transmission to other animals and humans. *Salmonella* are transmitted to animals through their environment, contaminated food, or their mothers before they are born or hatched (Banks *et al*., 2010). *Salmonella* are found in the intestines of a most of poultries. *Salmonella* infected animals excrete the bacteria in their feaces, which can easily contaminate their body parts (fur, feathers, or scales) and everything in the environment where they live and stay. It's crucial to understand that many animals can hold *Salmonella* while appearing safe and clean.

Human salmonellosis is usually occurred from the consumption of undercooked meat and poultry, raw eggs and milk. Furthermore, humans may get *Salmonella* if they do not wash their hands after coming into contact with *Salmonella*-infected animals or their surroundings, such as their bedding, food, or tank water(Bolton *et al*., 2014). Also reptiles and amphibians living in tanks or aquariums can also contaminate the water with *Salmonella* bacteria, which can make people sick even though they don't touch the animal. It's crucial to consider the cleaning of food surface and personal hygiene routines, as both have a role in bacterial transmission. This is only one of the reasons why correct handling and cooking practices training is the most important for consumer safety. Human to human transfer is unusual in developed countries, but really does appear (Pavic et al., 2012).

Since *Salmonella* can live in the environment for a long time, it acts as an infection source in a contaminated environment. *Salmonella* is then spread to vectors such rats, flies, and birds, *Salmonella* can be found in their feaces for weeks or months. Following direct transmission, moving animals such as swine, cows, and chickens were recognized as a significant risk factor for infection. *Salmonella* usually comes from contaminated environments and contaminated feed, so these animal reservoirs are infected orally (Davies *et al*., 2004). Humans become infected with *Salmonella* when they intake infected food or drink contaminated water. However, because *Salmonella* Typhi and *Salmonella* Paratyphi A don’t have an animal reservoir, infection seen when infected human intake improperly handled food (Newell *et al*., 2010).

2.11. Treatment of Salmonella Infections

Treatment and preventive care are recommended for animals with systemic or septicemic disease. The treatment of asymptomatic intestinal *Salmonella* infection is debatable due to the risk of carriers or antibiotic resistance(Onwuezobe *et al*., 2012). In septicemic S*almonella* early treatment is significant, but the use of antimicrobial agents for intestinal salmonellosis is controversial. Oral antibiotics can be ineffective and can harm the intestinal microflora, intervening in competitive antagonism and prolonging the organism's removal. Antibiotic-resistant *Salmonella* strains that are treated by oral antibiotics can also infect people subsequently. Antibiotics can also support the

transformation from susceptibility to *Salmonella* by the suppression of antibiotic responsive parts of natural flora to resistant strains of *E coli*. In many countries the use of chemical antibiotics to stimulate growth is therefore prohibited (Sengupta *et al*., 2013).

Whenever septicemia with *Salmonella* is suspected, gram-negative antibiotics spectrum should be administered parenterally soon. The initial antimicrobial therapy should center on understanding the pharmacological tolerance pattern of the species in question. Nosocomial infections are possible with highly medication resistant organisms. Combinations of trimethoprim-sulfonamide can be effective in treating. Ampicillin, fluoroquinolones or cephalosporins of the third generation are alternatives. Ampicillin, trimethoprim, sulfonamide, tetracycline and aminoglycosides resistance is generally mediated by plasmid and easily transfers between various bacteria. Quinolone resistance is mutative but random mutations can be selected and transmitted through the use of antibiotics. The therapy should last up to 6 days daily (Eng *et al*., 2015). Because affected animals are thirsty due to dehydration and have a poor appetite, oral medication should be given in drinking water rather than mixed into solid feed. To correct acid-base imbalances, fluid therapy and dehydration may be required. Calves, adult animals, and horses all require a lot of water. Antibiotics such as ampicillin and cephalosporins cause bacterial lysis and the release of endotoxin, indicating the use of NSAIDs to mitigate the effects of end toxemia (Chamoun-Emanuelli *et al*., 2019).

In all species the intestinal form is hard to treat. Although a therapeutic cure is accomplished, it is impossible to bacteriologically cure the bacteria since either the cells develop themselves in the biliary system and are intermittently transmit into the intestinal lumen, or because at a point where their natural bacterial flora is destroyed through antibiotic treatment, which is inhibitory of pathogens colonization. Antimicrobial therapy concerns that it could increase the risk in subclinical animals; antimicrobial therapy prolongs the period after clinical recovery in humans and other animal species during which the pathogen were recovered from the GIT (Acheson and Hohmann, 2001).

2.12. Control of Salmonella Infections

The challenge implementing a *Salmonella* control program is to consider its wide range of epidemiology and the entire production system chain involved .Any control program must start with setting up a monitoring program and serotyping the isolates. We can design a control program once we know which serotype is commonly found in poultry and where the source is. Once the serotype has been determined, we must return to the beginning of the chain (breeders, hatchery, grow out, and feed) to obtain the isolates and serotype in order to locate the source. If the serotype is found in the breeders, the breeders should be the focus of our control efforts (Dhama *et al*., 2013). If *Salmonella* is not found in the day-old chick but is found in the feed, the focus of control should be on the feed rather than the breeders. We can sometimes identify multiple sources of infection; in this case, the control program must consider all of them. *Salmonella* control revolves around the successful combination of several strategies, including providing *Salmonella*-free day old chicks and feed to the farm, effective biosecurity to keep *Salmonella* out of the flock, managing the gut microflora so that it is best able to counter *Salmonella* infection (colonization), and vaccination. Then, on top of this, we must have a robust monitoring system that can tell us what the true *Salmonella* status of our flocks really is (Panel *et al*., 2019).

2.12.1. Hatching eggs and day old chickens

The cornerstone to any *Salmonella* control programme is to be able to regularly provide and place *Salmonella* free day old chicks and this necessitates *Salmonella* free breeder flocks and regularly monitoring these to confirm that this status is being maintained. Specifically we need to produce clean hatching eggs that are regularly collected from the nest boxes and then disinfected or fumigated (Wray , 2000). At breeder level there is a conundrum with vaccination; in that vaccination reduces the level and degree of *Salmonella* shedding from a flock so that this process can, conceivably, reduce the level of *Salmonella* to below the level where routine testing could detect it. On the other hand, vaccination greatly reduces vertical transmission. In practice this is addressed by vaccinating commercial parent stock flocks but, as we ascend the breeder pyramid, reducing the reliance on vaccination and placing more emphasis on feed management, biosecurity and other aspects of *Salmonella* control. Risk of spreading *Salmonella* from

an undetected positive breeder flock is further minimized by streaming the eggs from a flock. Ideally, enough eggs should be placed in a single stage incubator to provide enough chicks for one broiler house (Marín *et al*., 2011).

2.12.2. Controlling salmonella in feed

Salmonella enters the poultry farm mostly through feed, which is a well-known route. So, management has the responsibility of producing *Salmonella* negative feed and ensuring that its status is not compromised between the feed mill and the farm. There is not much point in spending a lot of time, effort and money to produce *Salmonella* negative feed only to go and put it in an open lorry that did not have its tarpaulin on overnight and has become contaminated by broilers chicken fecal (Jones, 2011). The key strategies to obtaining *Salmonella* free feed are to source known *Salmonella* free ingredients, heat treatment of the feed, chemical treatment of the feed by acids or formalin containing compounds and avoiding its recontamination after the feed has been produced (Binter *et al*., 2011).

2.12.3. Biosecurity

The overall management plan of poultry producing integrators and companies should include general hygienic and biosecurity measures. These measures are critical for infection control, and when the overall biosecurity plan isn't working, all other measures lose their effectiveness**.** Everything coming on to the farm has the potential to bring *Salmonella* with it and the closer that any item gets to the birds the greater the risk that the birds will be contaminated(Damiaans, 2020). For this reason only an essential item should be allowed into the poultry house and then only after it has been subjected to processes that will ideally eliminate, but more likely greatly minimize, the number of *Salmonella* organisms present on or in it. Feed and water present the greatest risk, closely followed by bedding material, as every bird eats and drinks every day and, in the case of bedding material, picks through it. In this context the practice of floor feeding warrants consideration because it encourages birds to scratch through the litter and increases the accidental consumption of litter. Water should come from a clean source and ideally should be chlorinated to prevent infection of *Salmonella* (Todd-Searle *et al*., 2020). For essential visitors, wearing protective clothing (coveralls, gloves, mask, etc)

and disinfected boots should be allowed on-farm. Simple measures such as foot baths, wheel baths, hand hygiene and minimizing movement between different animal houses need to be implemented properly. Cleaning and disinfecting buildings, surfaces, fans, cooling pads, and equipment are essential. Establish clear zone, free from vegetation, around building to discourage rodent and insect traffic in the building. Cleaning and disinfection after each production cycle must be routinely performed in an overall management plan.

2.12.4. Managing the gut flora

In order to infect or colonize the digestive tract of the bird *Salmonella* needs to occupy places (receptor sites) on the surface of the intestinal tract (Bäumler *et al*., 2000). If we can swamp the digestive bacteria with 'good bacteria' then these will occupy these sites and make them unavailable for *Salmonella* bacteria that will then not be able to colonize or infect the birds. This process is known as competitive exclusion and we can use this in poultry production. There are two times when the beneficial bacterial population in the bird's digestive tract is reduced. These have to take shortly after hatching and after antibiotic treatment. In these situations the use of a competitive exclusion product may be beneficial. In the day old chick additional protection can also be provided from maternal immunity acquired from vaccinating the breeder hen (Jarquin *et al*., 2007).

2.12.5. Vaccination

Vaccination at commercial level is an important control tool, especially to control infections by *Salmonella enteritidis* and *S. typhimurium* (and for controlling pullorum disease and fowl typhoid in areas where these are still a problem). Vaccines tend to cross-protect within serogroups, which has an unstated advantage. As a result, the *S. typhimurium* vaccine protects against not only *S. typhimurium* but also other serogroup *B salmonella*e. The same occurs with *S. enteritidis* and other serogroup D *Salmonella*e. Vaccination has two purposes. Firstly, it protects the vaccinated birds against infection and, in doing this, greatly reduces horizontal (bird to bird) and vertical (bird to egg and hence to chick) spread (Van Immerseel *et al*., 2005). Secondly, as mentioned earlier, if we vaccinate breeders we confer protective maternal immunity on to the chicks produced and various kinds of vaccine are available. All of these strategies must be balanced in

order for the best Salmonella control programs to be implemented. Poor programmes place too much emphasis on one of the components. In essence, *Salmonella* control is like a chain it is only as strong as its weakest link (Mani-López *et al*., 2012).

2.13. Broiler Chicken Production in Ethiopia

Broilers chicken farming has a lot of potential in terms of creating jobs, improving family nutrition, empowering women (especially in rural areas), and, ultimately, ensuring household food security. Poor women are often the ones who engage in extensive scavenging broilers chicken production. It is popular for a number of reasons, including the low initial investment and the fact that it does not usually conflict with women's other household responsibilities. Broiler chickens are raised in houses, which are large open structures where they can roam, explore, feed, and socialize with other chickens (Gebremedhin *et al*., 2016). Broiler chickens take about seven weeks to reach market weight, and once they have reached the appropriate age and size, they will all be processed together. Broilers are housed in an industrial mode with a low to moderate bio-security standard in the commercial poultry production system, and all chickens are vaccinated against various chicken diseases. In about 40 % of the farms raising poultry under this system, the chickens are entirely confined indoors (CSA, 2017).

Key players in the broilers chicken sectors jointly produce yearly about 4.3 million broiler in Ethiopia (Alemu *et al*., 2020) The value chains of both the medium and large-scale intensive and family poultry production systems demonstrate that the industry faces significant challenges in terms of achieving optimal production productivity, feed availability and quality, and product and input marketing. In both systems, however, the poultry sector has bright prospects for development. Policy makers have shown a strong interest in and support for the industry (Ayele and Rich, 2010).

Poultry industry in central Ethiopia such as Bishoftu and Modjo towns plays a vital role in the small scale socio-economic system by contributing significantly on economic growth and simultaneously creating numerous employment opportunities these days (personal observation). In many developing countries, including Ethiopia, poultry plays an important role in the subsistence of poor rural households and periurban and urban areas. Small-scale semi intensive poultry farming has flourished in Ethiopia's urban and

per-urban regions in recent years. This is particularly prevalent in the Oromia towns of Modjo, Bishoftu and Adama. The majority of these small-scale poultry farms are situated in the compound or near to human residential, increasing the risk of infection transfer to humans. *Salmonella* is one of the common bacterial pathogens transmitted from poultry and poultry products to humans (Han *et al*., 2013).

2.14. Salmonella infections in chickens in Ethiopia

Because of the different chicken population rising in the country's and various agro-ecologies, determining the true picture of *Salmonella* in the country is challenging (Reta, 2009). Considerable differences in level of susceptibility to *Salmonella* have been reported within different poultry breeds. Perfect managing *Salmonella* in poultry production system is difficult due to the various possible sources of infection, which include chickens, feed, mice, wild birds, and poultry processing plant facilities, and workers (Kim *et al*., 2013) as well as hatcheries. Knowing where *Salmonella* comes from and how it spreads is one of the most important aspects of managing it. The Ethiopian agricultural research centers have established research-extension systems that work together to move improved technology products. (e.g. commercial poultry breeding, production and distribution centers) to end users (Abdi *et al*., 2017). In Ethiopia still exact distribution *Salmonella* infection in poultry breeding, multiplication, and centers is not well studied.

In Ethiopia, like other developing countries, it is difficult to evaluate the situation of *Salmonellosis.* This is mainly because of the very limited scope of studies, lack of coordinated epidemiological surveillance system and inadequacy of laboratory facilities for culture and identification of *Salmonella*. In addition, under reporting of cases and the presence of other diseases considered to be of high priority may have over shadowed the problem of salmonellosis in some countries, including Ethiopia. Nonetheless, considering the high prevalence of HIV/AIDS malnutrition, aggravated by recurrent drought, the general poor sanitary conditions, home slaughtering of food animals in the absence of meat inspection, feeding habit of people (consumption of raw meat, undercooked and some raw internal organs), lack of chilling facilities and wide spread occurrence of *Salmonella* in animals, it is expected that the disease to be common in Ethiopia, particularly among young, elderly and immune compromised citizens

(Birhaneselassie and Williams, 2013). The few studies conducted thus far are given in Table 1 below.

Table 1: The prevalence of *Salmonella* isolates form various Animal species in Ethiopia

Author	Study Area	Sample	Sample size	Number of Isolate
Assefa (2021)	Adama	Meat	66	4(6.1)
Zelalem *et al*(2019)	Dukem	Meat	118	15(12.7)
Tesfaw *et al* (2013)	A.A Dairy Product	Dairy product	384	6(1.6)
Mulaw (2017)	Dairy Farm of Bahir Dar	Milk	384	36(9.4)
Abebe *et al* (2020)	Tigray Woredas	Meat	384	63(16.4)
Tadesse (2012)	Kersa district,Jima	Milk	100	20(20)
Abate *et al* (2013)	Sebeta	Milk	100	16(16)
Assefa *et al* (2011)	Kombolcha	Egg	400	46(11.5)
Tsegaye *et al* (2016)	Alage,Ziway,Shshamane	Egg	392	52(13.3)
Taddese *et al* (2019)	Jima town	Egg	415	11(2.7)
Ejo *et al* (2020)	Gonder	Feed	384	21(5.5)
Fisseha (2015)	Dire Dawa town	Meat	290	8(2.8)
Addis *et al* (2011)	AA dairy farm	Milk	195	6(3.1)
Ali *et al* (2020)	Debrezeit and Modjo	Meat	384	56(14.6)
Kebede (2018)	AA Abattoir Enterprise	Meat	280	13(4.64)
Hunduma et al (2019)	Borane,SouthernEthiopia	Milk	150	7(4.7)

3. MATERIALS AND METHODS

3.1. The Study Area

The study was conducted in broiler farms located in Bishoftu and Modjo towns, central Ethiopia. Bishoftu is located between 8°43’ north – 8°48' North latitude and 38°00' east – 38°48' East longitude in the Regional State of Oromia, Ethiopia, approximately 47 km southeast of Addis Ababa (figure 2). Its topography is undulating, with flat land to the north and east of the town, which is surrounded by many lakes, and hills to the south (Peal *et al*., 2020). The total land area of the town is about 15,273 ha, and it lies at an altitude in the range of 1900 m to 1995 m above sea level. The town is featured by its well-developed poultry and dairy productions.

Mojo (also spelled Modjo) is a town in central Ethiopia that takes its name from the nearby Modjo River Situated in the East Shewa Region of the State of Oromia, it is situated at 8º39′N latitude and 39°5′E longitude with an average elevation ranging from 1788 to 1825 meters above sea level at about 70 Km south east of Addis Ababa.

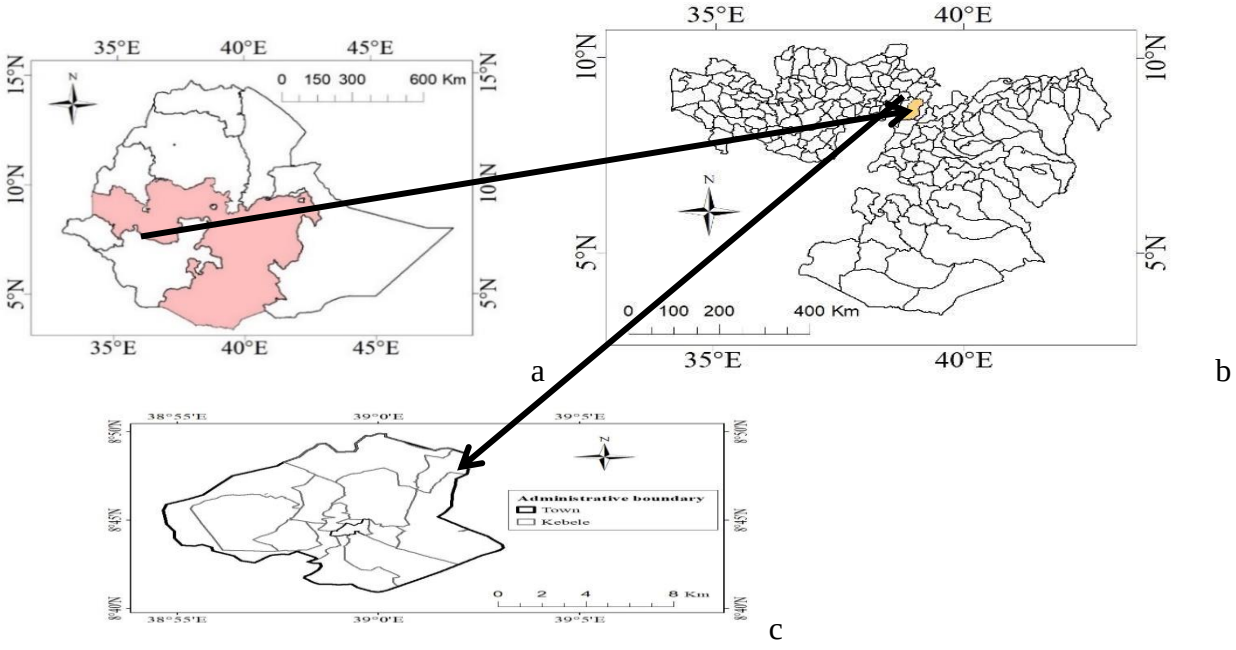

Figure 2: location map of the study area :(a) Regions of Ethiopia, (b) Weredas of Oromia regional state, (c) Bishoftu town

3.2. Description of the Farms and Slaughter House Studied

The samples (cloacal swabs, feed and water samples) used for isolation of *Salmonella* used in this study were collected from smallholder broiler farms located in Bishoftu and Modjo towns whereas the contents of the cecum were collected from chicken slaughtered at Chico Meat slaughter house in Bishoftu. Chico Meat established by Jacobs's integrated farm, has been distributing day old broiler chickens to smallholder farmers in Bishoftu, Modjo and Adama. The smallholder farms serve as out growers and supply the broilers to the slaughter house established in Bishoftu. The farmers were provided with day old chickens, feeds and other inputs, which were discounted when the chicken were provided to the Chico Meat slaughter house. The farmers use similar management practices they obtained from the Chico Meat team and all provide the finished chicken to the slaughter house. The slaughter house is a modern slaughter house with processing capacity of 10,000 kg a day or 25,000 chickens a week. The slaughter house has established link with local restaurants, hotels, supermarkets, resorts and others retailers from almost all parts of Ethiopia. The Chico Meat distributes Ross 308, Hubbard efficiency plus and Cobb 500 broiler chicken imported from Belgium, France and Netherlands. In the studied small holders broilers chicken modest flock sizes were usually ranging from 500 to 2500 are kept for 35-50 days till they reach for slaughter.

3.3. Study Design

From November 2020 to May 2021, a cross-sectional study was undertaken on small holders Broilers chicken farms located in Bishoftu and Modjo and slaughter house located in Bishoftu. The research was conducted to investigate the epidemiology of *Salmonella* in broilers chicken in selected farms and their inputs (feeds and water). The farms were selected purposively since they are parts of the small scale farmers (out growers) operating under the auspices of Chico Meat. At present there are more than ten smallholder broiler farms under the auspices of Chico Meat in Bishoftu and Modjo, of which four were selected for this study. Hence two farms designated B1 and B2 were selected randomly from Bishoftu and M1 and M2 were similarly selected from Modjo.

3.4. Sample size determination and Sample Type

Since there was no the prevalence reported only in broilers chicken small scale in previous studies , sample size was calculated using 5% desired absolute precision at 95 percent confidence interval with the suggested formula (Thrusfield, 2005).

$n = Z^2$ pexp (1-exp) /d2 Where n = required sample size; pexp = expected prevalence and a desired absolute precision (d) of 0.05, Z = 1.96. This was 384 samples to be collected from four selected farms. The actual collected sample size for each study were total 389 from which 189 cloacal samples, 52 feed samples, 48 water samples and 100 cecal contents were collected. From each of the farms selected in Bishoftu (B) 50 chickens were sampled (cloacal swab samples collected) making a total of 100 cloacal swab samples. From farm B1 14 feed samples of about 5 grams each and 11 water samples were collected. From farm B2 16 feed samples and 9 water samples were collected. From the two farms selected from Modjo (M) 89 cloacal samples (44 from M1 and 45 from M2) were collected. In addition, 11 feed samples and 14 water samples were collected from each of M1 and M2 farms for laboratory analysis. Besides, these samples 25 chicken were randomly selected at slaughter house from each of these farms and cecal contents collected for isolation and identification of *Salmonella*. The cecal samples were collected from those flocksrandomly selected among those from which the cloacal swabs were collected.

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Questionnaire survey: A total of 8 respondents (4 owners or farmers and 4 from feeders and other workers) were participated in the survey. Purposive sampling technique and structured questionnaires were used to gather information (Annex 1). The data obtained were used to evaluate the awareness and knowledge of the respondents about *Salmonella* infection in small scale poultry farms.

3.5. Sampling Method

Both wings of the broiler chickens were held with one hand keeping their heads down so as to expose the caudal portion upwards. Before collection of samples the cloaca surface was disinfected by using 70 % alcohol for 2 minutes. Sterile cotton swab was moistened and inserted into the cloacae and rolled inside several times. The cecal contents 0.5-1 gm were placed (squeezed) in standard universal tubes, each containing 10 ml of

buffered peptone water (Meteab and Abed, 2018) . Feed through 10-15 gm from each feeders were also collected into universal tube .The prepared Sterile cotton swabs were inserted into the each water drinker prepared in farms of broilers and collected in to test tubes. Aseptic procedures were followed when collecting samples where sterile plastic bags or cotton buds and sterile ice box were used. The all samples were individually labeled placed in separate plastic bags and transferred into sterile ice box and transported to the Microbiology Laboratory, College of Veterinary Medicine and Agriculture, Addis Ababa University for isolation of *Salmonella*.

3.6. Isolation of Salmonella

The isolation of *Salmonella* was conducted using the conventional bacteriological methods described for detection and isolation of *Salmonella* species in ISO guidelines (ISO, 2007a and b). For cultural isolation of *Salmonella*, a three-stage process namely pre-enrichment phase, selective enrichment phase and selective plating phase recommended by the International Organization for Standardization were employed (ISO, 2007a).

3.6.1. Pre-enrichment phase in non-selective liquid medium

In the pre-enrichment phase the samples were incubated at 37 ºC for 18-24 hours in buffered peptone water (BPW) and nutrient broth to allow *Salmonella* cells that have been sub-lethally injured are resuscitated and multiplied (Kim and Bhunia, 2008) and resuscitation and multiplication of sub-lethally damaged *Salmonella* cells (Blackburn, 1993).

3.6.2. Enrichment in selective liquid media

For this purpose the Rappaport Vassiliadis Medium with Soya was used as a selective enrichment medium for the detection of *Salmonella* spp. in cloacal swab samples, cecal contents, animal feed and water samples (Hammack *et al*., 2001). Rappaport Vassilliadis medium with soya (RVS) broth was inoculated with samples from pre-enrichment medium and incubated at 37.5 ºC ± 1 ºC for 24 hours ± 3 hours (Blackburn, 1993;

Varnam and Evans, 1991). The growth of *Salmonella* was monitored and the results recorded as evident by color change annex 7 (Figure 5).

3.6.3. Selective plating phase

For this purpose the bacteria that grow on enriched media were plated onto *Salmonella-Shigella* agar (SS agar), Xylose-Lysine Deoxycholate agar (XLD) and Brilliant Green Agar (BGA) as described by ISO 6579-1:2017 (Pol-Hofstad and Mooijman, 2019). First the samples from enriched medium were plated onto SS agar and incubated at 37 ºC ± 1 ºC for 18-24 hours and were examined for the presence of suspected *Salmonella* colonies. The pure *Salmonella* colonies from SS agar were sub- cultured onto XLD agar and BGA. *Salmonella* is not lactose ferment, but produce hydrogen sulfide (H_2S) gas. The incubated media were monitored for the presence various colors indicating the growth *Salmonella* as shown in annex 7 figure 6 A and B.

3.6.4. Gram's staining

The typical *Salmonella* colonies from XLD and BGA were collected onto glass slide, smear prepared, fixed and stained with Gram's stain as described by (Pierce and Gates, 1973). The stained smear was examined under microscope using oil immersion (Quinn et al., 2002b) annex 7as shown in figure 7.

3.7. Biochemical Characterization

Typical *Salmonella* colonies were further characterized by biochemical test such as triple sugar iron (TSI) agar slants (OXOID, Basingstoke, England), lysine decarboxylase test using lysine decarboxylase broth (DIFCO, Becton, Dicknson, USA), Methyl Red Voges Proskauer Broth (MRVP), Simmons Citrate Agar, Sulfide Indole Motility Medium (SIM), indole test and urease test using urea broth (HIMEDIA, Mumbai, India) according to the procedures describe by Quinn *et al*., (2002). For TSI test samples from typical colonies were inoculated onto TSI slant about 2/3 of the way into the butt, incubated aerobically at 35-37 °C and examined after 18-24 hours as described previously (Andrews *et al*., 2011) annex 7 (figure 8). The Lysine Decarboxylase test was carried out by inoculation of the suspected colonies of

Salmonella into Lysine Decarboxylase Broth and incubation for 4 days while being examined every day. Depending on whether the color of the medium changed to yellow and remains so or reverted to purple, differentiation of the isolates into S. typhi A or others can be achieved by this (Møller, 1955) annex 7 as shown in figure 9. The utilization of citrate by the *Salmonella* organisms was tested on Simmons Citrate agar in which dipotassium phosphate was used as a buffer. The incubation conditions and time was followed as described by (Timm, 1976) and as describe by this author based on the color pattern produced this test was used to distinguish between citrate positive *Salmonella* enteritidis and citrate-negative bacteria *Salmonella* typhi, *Salmonella* paratyph*i* , *Salmonella* pullorum, and *Salmonella* gallinarum (Timm, 1976) annex 7 (figure 10). Other biochemical tests such as the Sulfide Indole Motility, the Methyl Red test and Voges-Proskauer test were conducted following standard procedures (Lee *et al*., 2003).

3.8. Antimicrobial susceptibility Tests

Antimicrobial susceptibility of *Salmonella* isolates were conducted using standard Kirby-Bauer disk diffusion technic based on the guidelines of Clinical Laboratory Standards Institute CLSI (2013). Bacterial inoculate were prepared by suspending the freshly grown bacteria in sterile nutrient broth and the turbidity was corrected to a 0.5 McFarland standard and using a sterile cotton swab, gently spread over the Muller-Hinton Culture plate. Eleven antimicrobials that have been commonly used in poultry practices and treatment of human cases of salmonellosis were used for this assay. They include: Gentamycin (10 µg), Erythromycin (15 µg), Streptomycin (10 µg), Chloramphenicol (30 µg), Sulphamethazole/Trimethoprim (25 µg), Tetracycline (10 µg), Ampicillin (10 µg), Cloxaciline (5 µg), Naldixic acid (30 µg), Amoxicillin (25 µg) and Kanamycin (30 µg). The medium containing the bacterial inocula and the antibiotic disks were incubated aerobically at 37 °C for 18–24 hours and zone of inhibition around each antibiotic disc annex 7 (figure 11) was determined by using transparent ruler to classify the organisms as sensitive, intermediate or resistant as indicated by CLSI (2013) annex 5.

3.9. Data Management and Analysis

To describe the nature and characteristics of the data, descriptive statistics such as percentage and frequency distribution were used. The effects of various risk factors on the isolation of *Salmonella* were analyzed by logistic regression using R where the strength of association was expressed using odds ratio (OR) (annex 5). The probability of type one error was set at 5% with 95% confidence level to establish biological and statistical association between dependent and independent variables.

3.10. Ethical Considerations

Ethical approval for this study was obtained from the Ethics Review Committee of the College of Veterinary Medicine and Agriculture, Addis Ababa University (Bishoftu) with Certificate Ref No: VM/ERC/16/05/13/2021 (Annex 8). Moreover, all participants included in the study were informed of the study objectives and informed oral consent was taken from each one of them.

3.11. Limitations of study

This research was initially proposed to detect molecularly characterizing salmonella serotypes. On this Addis Ababa University college of Veterinary Medicine and Agriculture assisted by writing letter to molecular analyzer laboratories but due to lack of reagents within analyzer laboratories we couldn't come with molecular result.

4. RESULTS

4.1. Prevalence of Salmonella in the samples

A total of 389 samples were collected and cultured for isolation of *Salmonella* from broilers chicken farms. Over all 99 samples were positive results yielding a prevalence of 25.45 % (95 % CI: 21.19, 30.08). Of the samples collected from Bishoftu 21.50 % (43/200; 95 % CI: 16.02, 27.85) were positive whereas of the 189 (29.63 %; 95 % CI: 23.22, 36.69) samples collected from Modjo were found positive as described in table 2.

At the farm level 18.00 % (95 % CI: 11.03, 26.95), 25.00 % (95 % CI: 16.88, 34.66), 30.85 % (95% CI: 21.73, 41.22) and 28.42 % (95 % CI: 19.64, 38.59) of the samples collected from B1, B2, M1 and M2, respectively gave positive results for *Salmonella*. As presented in Table 3 were 26.46 % of the cloacal samples, 21.00 % of the cecal contents, 30.77 % of the feed samples and 25.00 % of the water samples yielded *Salmonella*. The differences observed among farms and samples were, however, not significant.

Table 2: Prevalence and distribution of *Salmonella* in Bishoftu and Modjo town

Place of sample	N	*Salmonella*(+)	Prevalence	95% CI	P-value	Chi-square
Bishoftu	200	43	21.50%	16.02, 27.85		
Modjo	189	56	29.63%	23.22, 36.69	0.07	3.38
Total	389	99	25.45%	21.19, 30.08		

N-number

Table 3: Prevalence of *Salmonella* from each sample and selected farms

Type of sample	N	*Salmonella* (+)	Prevalence	95% CI	Chi-square	P-value
Cloacal swab	189	50	26.46%	20.32,33.35		
Cecum content	100	21	21.00%	13.49,30.29		
Feed	52	16	30.77%	18.72,45.10	1.92	0.59
Water	48	12	25.00%	13.64,39.59		
Name of farm						
B1	100	18	18.00%	11.03, 26.95		
B2	100	25	25.00%	16.88, 34.66	4.82	0.19
M1	94	29	30.85%	21.73, 41.22		
M2	95	27	28.42%	19.64, 38.59		

B-Bishoftu M-Modjo N-Number

4.2. Univariable Logistic Regression Result

The effect of potential risk factors on the occurrence of *Salmonella* was assessed using Univariable logistic regression with odd ratio as a measure of association. The results showed that the likely hood of isolation of *Salmonella* was times 0.38 and 0.39 lower from Ross-308 than Hubbard classic and Cobb 500.The difference seen among others risk factors were not significance (table 5). Univariate analysis commands were used annex 6(2).

4.3. Multivariable Logistic Regression Result

Higher proportion of samples collected from chicken in the age range of 21-30 days (28.47 %) yielded Salmonella than younger ones (20.00 %). Higher proportion of samples collected from Cobb 500 (28.57 %) and Hubbard classic (28.47 %) gave positive results for Salmonella whereas lowest proportion was observed in samples collected from Ross-308 (13.33 %) and the differences observed among risk factors were not significant (Table). Multivariable analysis commands were indicated in annex 6(1).

Table 4: Univariable logistic regression results

Risk factors		OR	95% CI	p-value
Age (days)	21-30	---	---	---
	31-40	0.68	0.36,1.24	0.21
	41-45	0.93	0.41, 2.20	0.87
Breed	Ross-308	---	---	---
	Hubbard Classic	0.39	0.17, 0.80	0.01
	Cobb500	0.38	0.16, 0.88	0.03
Source of Water	Tap water	---	----	----
	Tank water	1.34	0.78, 2.30	0.30
Type of house	Wire round house	----	---	---
	Cement block	1.25	0.71, 2.18	0.44
Farm location	Residential compound	---	---	---
	Separate	0.85	0.47, 1.45	0.48

Table 5: Multivariable logistic regression results

Risk factors		*Salmonella* tested					
		n-tested	(+)	Prevalence	OR	95% CI	p-value
Age (days)	21-30	95	19	20.00%	---	----	----
	31-40	144	41	28.47%	0.30	0.03,2.43	0.28
	41-45	50	11	22.00%	0.50	0.01, 13.68	0.68
Breed	Ross-308	75	10	13.33%	---	----	---
	Hubbard classic	144	41	28.47%	0.76	0.081, 8.16	0.88
	Cobb 500	70	20	28.57%	0.42	0.01, 14.27	0.63
Source of Water	Tap water	146	32	21.92%	---	---	----
	Tank water	143	39	27.27%	0.42	0.11, 1.60	0.20
Type of house	Wire round house	194	45	23.20%	---	---	---
	Cement block	95	26	27.37%	2.77	0.10, 91.47	0.56
Farm location	Residential compound	145	41	28.28%	---	---	---
	Separate	144	30	20.83%	0.64	0.20, 2.04	0.44

4.4. Results of Questioners Survey

The awareness of the farm owners or attendants on the occurrence of *Salmonella* infection in poultry was assessed using questionnaire on 8 randomly selected individuals. The results showed that the farmers or attendants did not have knowledge on the occurrence and impacts of salmonellosis. However, they use the necessary biosecurity measures aimed at reduction of occurrence of various diseases (Table 6).

Table 6 : The results of questionnaire survey on the awareness and knowledge, of farmers and feeders on *Salmonella* infection

Educational back ground	Illiterate	Educated
Farmers(owners)	1(25%)	3(75%)
Feeder	4(100%)	0(%)
Questioners	Yes	No
Do you use foot bath	8(100%)	0(0%)
Use safety closes only for farm time	8(100)	0(0%)
Is broiler production your main economic activity?	4(50%)	4(50%)
Do you disinfect the chicken house before introduction next butch	8(100%)	0(0%)
Can you identify diseases in your farm by looking at clinical sign	0(0%)	8(100%)
Do you know risk of salmonellosis infection on the broilers?	0(0%)	8(100%)
Do you know the impact of *salmonella* infection on human health?	0(0%)	8(100%)
Do you take any control measure to prevent *Salmonella*?	Any diseases 8(100%)	0(0%)
Do you use prevention medicine on broilers	8(100%)	0(0%)
Have you received training or guidance on preventing diseases in your farm?	4(50%)	4(100%)

4.5. Antimicrobial Resistance Profiles of the Isolates

Ninety nine *Salmonella* isolates were tested for their antimicrobial susceptibility test profiles. The higher level of resistance (80 isolates, 80.81%) was observed against Tetracycline followed by Kanamycin (71 isolates, 71.72%) and Chloramphenicol and Amoxicillin (67 isolates, 67.68%) whereas most of the isolates were susceptible to Gentamicin (69 isolates, 69.70%) and Erythromycin (40 isolates, 40.41%). In general the *Salmonella* isolates obtained from this study showed resistance to various antibiotics commonly used in poultry practices (Table 7). Similar pattern was observed the *Salmonella* isolates were tested against the antibiotics according to the different samples (Table 8).

Table 7: Results of antibiotic susceptibility profiles of the *Salmonella* isolates against 11 different antibiotics

Antibiotics	Resistance (%)	Intermediate (%)	Susceptible (%)
Gentamycin (10 μg)	10(10.10)	20(20.20)	69(69.70)
Erythromycin (15 μg)	24(24.24)	35(35.35)	40(40.41)
Streptomycin (10 μg)	59(59.60)	24(24.24)	16(16.16)
Chloramphenicol (30 μg)	67(67.68)	17(17.17)	15(15.15)
Sulphamethazole/ Trimethoprim (25 μg)	61(61.62)	22(22.22)	16(16.16
Tetracycline (10 μg)	80(80.81)	16(16.16)	3(3.03)
Ampicillin (10 μg)	54(54.55)	23(23.23)	22(22.22)
Cloxaciline (5 μg)	29(29.29)	27(27.27)	43(43.44)
Naldixic acid (30 μg)	63(63.64)	23(23.23)	13(13.13)
Amoxicillin (25 μg)	67(67.68)	19(19.19)	13(13.13)
Kanamycin(30 μg)	71(71.72)	18(18.18)	10(18.18)

Table 8: Antibiotic susceptibility profiles of *Salmonella* isolates obtained from different samples

Antibiotics	Resistance *salmonella* on different type samples			
	Cloacal swab (%) (n=50)	Cecum content (%) (n=21)	Feed (%) (n=16)	Water (%) (n=12)
Gentamycin (10 µg)	6(12.00)	2(9.23)	0(0.00)	2(16.67)
Erythromycin (15 µg)	13(26.00)	3(14.30)	4(25.00)	4(33.33)
Streptomycin (10 µg)	30(60.00)	13(61.90)	8(50.50)	8(66.67)
Chloramphenicol (30 µg)	32(64.00)	14(66.67)	10(62.50)	11(91.67)
Sulphamethazole/ Trimethoprim (25 µg)	30(60.00)	15(71.42)	7(43.75)	9(75.00)
Tetracycline (10 µg)	43(86.00)	16(76.19)	11(68.75)	10(83.33)
Ampicillin (10 µg)	27(54.00)	13(61.90)	9(56.25)	5(41.67)
Cloxaciline (5 µg)	13(26.00)	7(33.33)	6(37.50)	3(25.00)
Naldixic acid (30 µg)	33(66.00)	13(61.90)	8(50.50)	9(75.00)
Amoxicillin (25 µg)	34(68.00)	13(61.90)	11(68.75)	9(75.00)
Kanamycin(30 µg)	36(72.00)	16(76.19)	10(62.50)	9(75.00)

In this study, 99 isolates were found to be resistant to at least two antibiotics. Fifty five of the 99 *Salmonella* isolates examined were resistant to two to five antibiotics, fourteen were resistant to six to seven and three were resistant to eight to ten antibiotics. All of the isolates tested were resistant to at least two or more antimicrobial agents examined (table 9).

Table 9: Results showing multiple antimicrobial resistance profiles of *Salmonella* isolates

Number	Antimicrobial resistance pattern	No of isolate resistant (%)
Two	GEN , ERY (3) AMO , KAN(52)	55(55.56)
Three	ERY,STR,CHL (12) SUL,TET,AMP (24) CHL,NAL,AMO (13)	49(49.5)
Four	TET,AMP,CLO,NAL (7) STI,CLO,SUL,TET(20) GEN,ERY,SRT,CHL(1) SUL,TET,AMP,CLO(7)	35(35.4)
Five	AMP,CLO,NAL,AMO,KAN(7) ERY,STR,CHL,SUL,TET(8) CHL,SUP,TET,AMP,CLO(6) TET,AMP,CLO,NAL,AMO(4) STR,CHL,SUL,TET,AMP(12)	35(35.4)
Six	ERY,STR,CHL,SUL,TET,AMP(3) STR,CHL,SUL,TET,AMP,CLO(4) CHL,SUL,TET,AMP,CLO,NAL(4) SUL,TET,AMP,CLO,NAL,AMO(3)	14(14.14)
Seven	STR,CHL,SUL,TET,AMP,CLO,NAL(3) CHL,SUL,TET,AMP,CLO,NAL,AMO(3) SUL,TET,AMP,CLO,NAL,AMO,KAN(3)	9(9.09)
Eight	STR,CHL,SUL,TET,AMP,CLO,NAL,AMO(3)	3(3.03)
Nine	STR,CHL,SUL,TET,AMP,CLO,NAL,AMO,KAN(3)	3(3.03)
Ten	ERY,STR,CHL,SUL,TET,AMP,CLO,NAL,AMO,KAN(3)	3(3.03)

Erythromycin (ERY), Streptomycin (STR), Chloramphenicol (CHL), Sulphamethazole/Trimethoprim (SUL), Tetracycline (TET), Ampicillin (AMP), Cloxaciline (CLO), Naldixic acid (NAL), Kanamycin (KAN).

5. DISCUSSION

Broiler meat production remains an important economic that significantly contribute to the food and nutrition security. However, in the absence of regular monitoring and surveillance broiler meat can be source of zoonotic pathogens. This study revealed that the occurrence *Salmonella* in broilers and their inputs in smallholder broiler farms in Bishoftu and Modjo towns. *Salmonella* were isolated from cloacal swabs collected from broilers on farms, cecum contents at slaughter house, feed and water samples. This shows widespread occurrence of *Salmonella* along the broiler value chain and has an important implications for public health. The overall proportion of samples yielding *Salmonella* in this study is comparable to the reports of 23.2 % in cloacal swabs from Asossa And Bambasi towns, (Asmamaw *et al*, 2018) and the 28 % isolation from broiler faces feaces from Senegal (Cardinale *et al*., 2004). However, our observation is higher than the reports of most of the previous studies conducted in Ethiopia including that of Ali *et al*. (2020) from Bishoftu and Modjo, Abunna *et al.* (2016) from Modjo, Aragaw *et al*. (2010) from Hawassa, Abdi *et al*. (2017) from central and southern Ethiopia and Bekele and Ashenafi (2010) in Addis Ababa in central Ethiopia.

Our findings are lower than the results of some studies carried out in Ethiopia and elsewhere in the world. For instances, higher proportion than our observation was reported in chicken from Jimma town, western Ethiopia (Kindu and Addis, 2013). Similarly higher proportion of *Salmonella* positive samples were reported by Edel (1994) in Netherlands, Ebel *et al*. (1992) in United States of America and Ishihara *et al*. (2009) in Japan. These differences in the proportion of samples yield *Salmonella* between our study and the previous ones could be difference in isolation techniques used, and difference in geographical locations, difference in biosecurity measure like cross-contamination and poor housing system, breed, age and source of water.

Relatively higher proportion of samples from Modjo was positive for *Salmonella* than Bishoftu. This could be due to the difference in experience in poultry management among owners from Modjo and Bishoftu. Broiler farmers in Bishoftu had some training and experience from other poultry farmers since commercial poultry farms have long been established in town. In contrary most of the smallholder broiler farmers in Modjo

were engaged in the business recently and lack experience and training. with the effect of poor management specially poor biosecurity practices were incriminated as *Salmonella* infection causes (Liljebjelke *et al*., 2005) and Thus the potential of *Salmonella* contamination is higher on farms with poor management(Huneau-Salaün *et al*., 2009). The widespread occurrence of *Salmonella* in all samples tested (water, feeds, cloacal swabs and cecal contents) coupled with the poor knowledge of the owners and attendants could have contributed to the transmission of the bacteria among flocks and probably among the farms. This is evident by the fact that the smallholder broiler farmers (out growers) included in this study share farm implements and other inputs among themselves. The hygienic status of the farms studied was also poor and it could be one of the factors that contributed to increased risk of contamination. The use of unclean Feeding materials and unhygienic houses were shown to be source of contamination by *Salmonella* in poultry (Davies and Hinton, 2000).

The results of this study revealed that higher proportion of samples collected from older broilers than from younger chicks suggesting the greater chance getting *Salmonella* from the environment increase with age. Although the results of some studies are in consent with our results (Gast and Beard, 1989) and (Beal *et al*., 2004) it contradicts the results of few of the previous studies such as that of Beal *et al*. (2004) who reported higher prevalence of *Salmonella* in young chicks. In this study higher proportion of samples collected from Cobb 500 gave positive results than Hubbard classic and Ross 308. This could be probably due to variation in the compatibility of the breeds in clearing the infections. Difference in the proportion of samples yielding positive results was observed between the houses types used to rear the broilers although it not statistically significant. It was higher in houses made of cement blocks than those constructed from round wire houses. This could be due to the possibility accumulation of waste and leftovers of feeds and water on the floor. Since the cement blocks do not allow circulation of sufficient air the water and waste materials excreted from the chicken create wetness on the floor, which is conducive for multiplication and survival of *Salmonella*. In contrast, the wire mesh allows the circulation of air reduces humidification and then the risks of *Salmonella*. The effects of housing type on the occurrence of *Salmonella* in poultry have been documented elsewhere. Farm located in residential compound were showed higher prevalence than separate and these difference could be due to load of contamination is higher in farms located in residential. From

factors tested for associations with occurrence of *salmonella*; breeds were shown significant difference in univariable analysis.

The results of questionnaire survey showed that all farmers and attendants did not have knowledge about the occurrence of *Salmonella* in broilers. This was evident in their inability to identify none of the signs of *Salmonella* infection in poultry, its transmission mechanisms and whether *Salmonella* spreads from poultry to humans. All of the participants did not have any information on the occurrence of *Salmonella* in broilers although they have good knowledge on other diseases of poultry. This could contribute the spread of *Salmonella* with in a farm or among farms and this will pose a health risk among the consumers broiler meat and attendants of the chicken. The trainings given to some of the smallholder farmers also did not contain *Salmonella* infection and rather focuses on other infectious diseases. In general the broiler farms lack awareness about *Salmonella* in poultry and this could risk the health of the community unless otherwise attempts will be made to reduce its occurrence.

The antibiotic susceptibility test results revealed that the *Salmonella* isolates obtained in this study were resistant to most the commonly used antibiotics/antimicrobials in poultry practices (Tetracycline, Kanamycin, Amoxicillin, Chloramphenicol, Nalidixic acid and Sulphamethoxin-trimethoprim). The use of these drugs has not been based on the prescription of the veterinarian. They have been used indiscriminately. This indiscriminate use of the drugs could have contributed to resistance shown by the *Salmonella* isolates. These antibiotics / antimicrobials are found in all veterinary drug vendors and on all farms. Empirical evidence obtained from some of the owners showed that these some of these drugs are also used as growth promoters. These drugs are also used widely in medical practices to treat infections with *Salmonella*. Hence, the occurrence of resistance against these drugs has important public health implications. The occurrence of resistance against Kanamycin, Sulphamethoxin-trimethoprim, Tetracycline, Nalidixic acid and Chloramphenicol reported in this study is consent with the findings of Fisseha (2015) and that of Su *et al*. (2004). This could be due to improper use of drug and uncontrolled availability of the antimicrobial agents in drug vendors, which leads to improper use. Thus, this might exert greater selection pressure for the resistant strains thereby making them resistant to antimicrobials (Addis *et al*., 2011; Tajbakhsh *et al*., 2012).

The presence of antimicrobial resistance have the potential to adversely affect human health by causing illness that is more difficult to treat because of the resistance profile of the microorganism. *Salmonella* serovars are becoming increasingly resistant to commonly used antimicrobials, which is a concerning trend. Antimicrobial resistance among *Salmonella* isolates is on the rise globally, most likely as a result of the widespread use of antimicrobial agents for the treatment of febrile syndromes (Bukitwetan *et al*., 2007).The highest level of susceptibility was found gentamycin (69.70%), Cloxaciline (43.44%), Erythromycin (40.41%), and Ampicillin (22.22%).

The present study revealed that, 72 (72.72 %) were multiple antimicrobial resistance isolates with different resistant pattern. Fifty five of the 99 *Salmonella* isolates examined were resistant to two to five antibiotics, fourteen were resistant to six to seven and three were resistant to eight to ten antibiotics and most of these isolates have developed resistance. The incorporation of antibiotics in the diet of animals at sub therapeutic concentrations as prophylaxes and as growth promoters has invariably contributed to the development of antibiotic resistant strains of *Salmonella* (Shah andand Korejo, 2012) In this study, the patterns of resistant to most antibiotics indicate the potential importance of broilers chicken as a source of multiple antimicrobial resistant to *Salmonella* infection and this is serious public health concern.

6. CONCLUSION AND RECOMMENDATIONS

The results of this study showed that *Salmonella* occur in poultry feed and water, in broilers grown under smallholder farms and their carcass at slaughter house in Bishoftu and Modjo and all type of samples gave positive results using bacteriological techniques. *Salmonella* were isolated from cloacal swabs, feed and water samples collected from smallholder broiler farms and cecal contents collected from slaughtered chicken at Chico-Meat slaughter house suggesting its widespread occurrence in broilers and their inputs. The results of questionnaire survey showed that all farmers and attendants did not have knowledge about the occurrence of *Salmonella* in broilers. The *Salmonella* isolated in this study were resistant to Tetracycline, Kanamycin, Chloramphenicol and Amoxicillin whereas most of the isolates were susceptible to Gentamicin and Erythromycin (40 isolates. The *Salmonella* isolates obtained from this study showed resistance to various antibiotics commonly used in medical and poultry practices bearing important implications to public health. Based on the results the following are recommended.

- Regular epidemiological investigation, monitoring and corrective measures are warranted
- Restrictions on the irrational use of antibiotics and establishment of standardized monitoring systems in Broilers chicken farms are required.
- Training of farmers and attendants is needed in poultry farms regarding sanitation, use of clean drinking water, promotion of safe food handling practices
- Awareness creation is needed on zoonotic *Salmonella* infection and the role bio-security
- *Salmonella* serotyping and molecular genotyping should be performed on the isolates obtained from this study

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8. ANNEXES

Annex 1: Questionnaire

These questions are used to collect some information about the Broiler Farm Management in Bishoftu and Modjo town of the respondents.

Farm name_____Address_____ Date _____

Respondent background information

Sex____ Qualification (field and certificate) _____

Age____ work and responsibility_____

1. Survey response of the poultry farmers and attendants

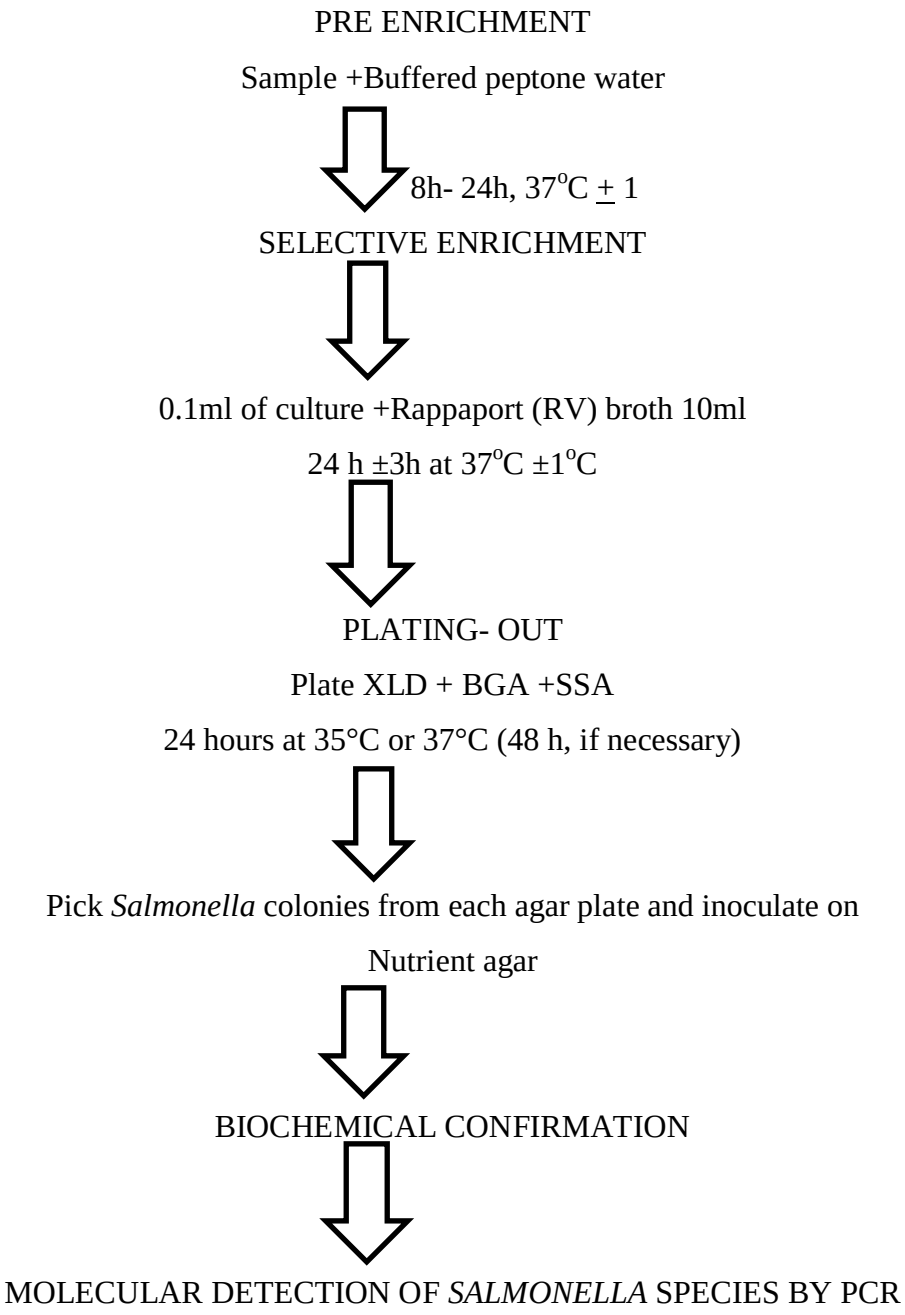
Put the yes or no in the box of your response.

Number	Question		
1	Is broiler production your main economic activity?		
2	Can you identify diseases in your farm by looking at clinical sign		
3	Do you know risk of salmonellosis?		
4	Do you know the impact of *Salmonella* on human health?		
5	Do you take any control measure to prevent *Salmonella*?		
6	Have you received training or guidance on preventing diseases in your farm?		

Annex 2: Records sheet of informations about farm and broilers history

Date of sample collection	Farm location	Age (days)	Type of house	sources of water	Breed	name of farm	type of sample

Annex 3**:** Flow diagram showing method for detection of *Salmonella*



Annex 4 : Culture media, reagent composition and preparation

1. Buffered peptone water

Composition (g/L)

Enzymatic Digest of Casein ……10.0

Sodium Chloride ……5.0

Disodium Hydrogen Phosphate …….3.5

Potassium Dihydrogen Phosphate …….1.5

Preparation: Suspend 20.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shacking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 min.

2. Rappaport Vassiliadis *Salmonella* Enrichment Broth

Composition (g/L)

Soy Peptone……. 4.5

Sodium Chloride…… 8.0

Potassium Phosphate, Monobasic……..0.6

Dipotassium Phosphate……..0.4

Magnesium Chloride, Anhydrous…… ……. 13.58

Malachite Green…….0.036

Preparation: Dissolve 28.00 g of the medium in one liter of purified water and mix thoroughly. Fill 10 mL glass tubes halfway with liquid, seal and autoclave for 15 minutes at 115°C

3. Xylose Lysine Deoxycholate (XLD) Agar

Composition of XLD Agar (g/L)

Lactose…….7.5

Sucrose……. 7.5

Sodium Thiosulfate……. 6.8

L-Lysine…….. 5.0

Sodium Chloride……... 5.0

Xylose …….3.75

Yeast Extract…… 3.0

Sodium Deoxycholate……2.5

Ferric Ammonium Citrate……..0.8

Phenol Red…… 0.08

Agar……15.0

Preparation: Suspend 56.8 grams of dehydrated medium in 1000 ml purified or distilled water. Heat with frequent agitation until the medium boils. Do not autoclave.

4. *Salmonella Shigella* Agar

Composition (g/L)

Beef Extract…….. 5.00

Enzymatic Digest of Casein……...2.50

Enzymatic Digest of Animal Tissue…….. 2.50

Lactose……...10.00

Bile Salts…… 8.50

Sodium Citrate…….. 8.50

Sodium Thiosulfate……8.50

Ferric Citrate…….. 1.00

Brilliant Green…….0.00033

Neutral Red……0.025

Agar…….....13.50

Preparation: Add 56.8.0 grams of Salmonella Shigella Agar in 1000 ml distilled water and heat to boiling to dissolve the medium completely. Mix well and pour into sterile Petri plates. Do not autoclave.

5. Triple Sugar Iron (TSI) Agar

Composition (g/L)

Beef Extract…….. 3.0

Yeast Extract……. ……..3.0

Peptone Mixture…….…. 20.0

Sodium Chloride……. ……5.0

Lactose…….. …….10.0

Sucrose…….. 10.0

Glucose…….. 1.0

Ferric Citrate…….. 0.3

Sodium Thiosulphate……....……. 0.3

Phenol Red ……..…….0.025

Agar……..... 12.0

Preparation: Suspend 65 g of the medium 1liter of distilled water. Heat with frequent agitation and boil to completely dissolve the medium and dispense into tubes. Autoclave for 15 minutes at 121°C and allows setting as a slope ensuring that the slant is over a butt approximately 3-4 cm deep.

6. Lysine Decarboxylase Broth M376

Composition (g / L)

Peptone……. 5.000

Yeast extract…… 3.000

Dextrose (Glucose)…… 1.000

L-Lysine hydrochloride …….5.000

Bromocresol purple……. 0.020

Preparations: Suspend 14.02 grams in 1000 ml purified / distilled water and then heat, to dissolve the medium completely. Dispense 5 ml amount into screw-capped test tubes. Sterilize by autoclaving at 121°C for 15 minutes. Cool the tubed medium in an upright position and overlay with 2-3 ml of sterile mineral o

7. Simmons Citrate Agar

Composition (g / L)

Magnesium sulphate…… 0.2

Ammonium dihydrogen phosphate…… 1.0

Dipotassium phosphate…… 1.0

Sodium citrate……...2.0

Sodium chloride ……5.0

Bromothymol blue…….. 0.080

Agar ……15.0

Preparations: Suspend 24.28 grams in 1000 ml purified/ distilled water and heat, to boiling, to dissolve the medium completely. Mix well and distribute in tubes or flasks and autoclave at 15 lbs pressure (121°C) for 15 minutes to sterilize.

8. SIM Medium

Composition (g / L)

HM Peptone ……..……3.0

Peptone ……..…….30.0

Peptonized iron …….…..0.2

Sodium thiosulphate…….. 0.025

Agar…… 3.0

Preparation: Suspend 36.23 grams in 1000 ml purified/ distilled water and heat to boiling to dissolve the medium completely. Dispense in tubes. Sterilize by autoclaving 121°C for 15 minutes. Cool the tubes to in an upright position.

9. Methyl Red (MR) Test (MRVP broth (pH 6.9))

Composition (g/L)

Buffered peptone…….…… 7.0

Glucose…….……5.0

Dipotassium phosphate…….5.0

Preparation: Dissolve 0.1 g of methyl red in 300 ml of ethyl alcohol, 95% and add sufficient distilled water to make 500 ml. Store at 4 to 8 ^{o}C in a brown bottle. Solution is stable for 1 year

10. Mueller Hinton Agar (MHA)

Composition (g/L)

Beef extract…… 2.0

Acid hydrolysate of casein…….17.5

Starch……. 1.5

Agar……17.0

Preparation: Dissolve 38.00 gm of the medium in 1Litre of distilled water; heat with frequent agitation for one minute to totally dissolve the medium, then autoclave at 121°C for 15 minutes. Pour cooled Mueller Hinton Agar onto sterile petri dishes on horizontal surface to obtain consistent depth. Allow to cool to room temperature before serving. Keep the plates between 2 and 8 ° C.

Annex 5: Performance standard for antimicrobial susceptibility testing of *Salmonella*

Antibiotics	Resistance (mm)	Intermediate (mm)	Susceptible (mm)
Gentamycin (10 µg)	≤ 12	(12-15)	≥ 15
Erythromycin (15 µg)	≤ 13	(13-18)	≥ 18
Streptomycin (10 µg)	≤ 11	(11-15)	≥ 15
Chloramphenicol (30 µg)	≤ 12	(12-18)	≥ 18
Sulphamethazole/Trimethoprim (25 µg)	≤ 10	(10-16)	≥ 16
Tetracycline (10 µg)	≤ 14	(14-19)	≥ 19
Ampicillin (10 µg)	≤ 13	(13-17)	≥ 17
Cloxaciline (5 µg)	≤ 10	(11-12)	≥13
Naldixic acid (30 µg)	≤13	14-18	≥19
Amoxicillin (25 µg)	≤13	14-17	≥ 17
Kanamycin(30 µg)	≤13	(14-17)	≥18

Annex 6: R analysis

1. Multivariable logistic regression

```
>Row_data <- read_excel("C:/Users/Administrator/Desktop/Row data.xlsx")
>attach (Row_data)
>multi_fit<-glm(Salmonella~factor(Breed) + factor(`Age (days)`) + factor(`farm location`) + factor(`tyep of samle`) + factor(`sources water`) + factor(`Type of house`),family = binomial(link = "logit"))
>summary (multi_fit)
>exp(coefficients(multi_fit))
>exp(cbind(OR=coefficients(multi_fit), confint(multi_fit)))
>anova (multi_fit, test = "Chisq")
anova(multi_fit, test = "LR")
>library (MASS)
>step <- stepAIC(multi_fit, direction = "both")
```

2. Univariable logistic regression

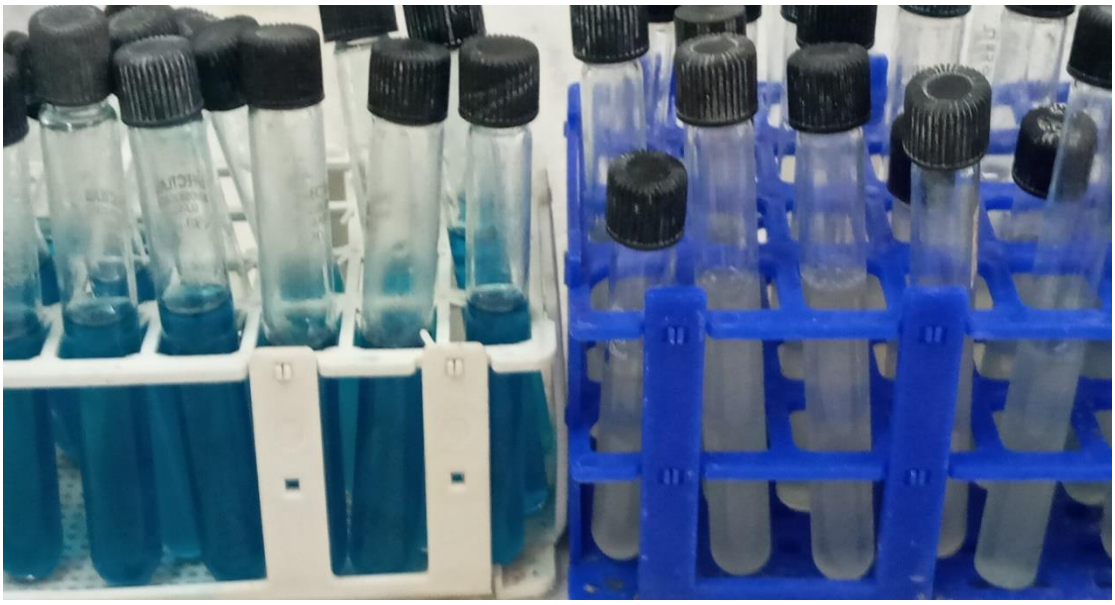
```
>age_fit<-glm (Salmonella~factor(`Age (days)`),family = binomial(link = "logit"))
>summary (age_fit)
>exp(coefficients(age_fit))
>exp(confint(age_fit))
>exp(cbind(OR=coefficients(age_fit), confint(age_fit)))

>Breed_fit<-glm(Salmonella~factor(Breed),family = binomial(link = "logit"))
>summary (Breed_fit)
>exp(coefficients(Breed_fit))
>exp(confint(Breed_fit))
>exp(cbind(OR=coefficients(Breed_fit), confint(Breed_fit))) and etc.
```

For all factors Univariable logistic regression commends were repeated for each

Annex 7: Preparations of testing Medias in laboratory, sample collections in slaughter house and biochemical tests results.

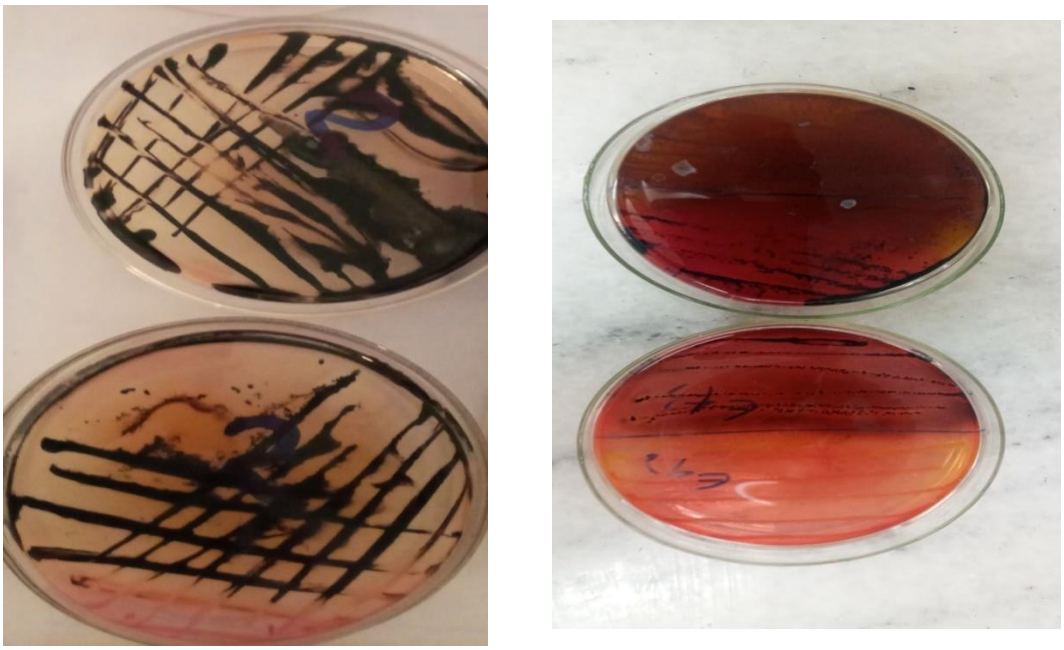
Figure 3: Preparation of Medias and reagents for *salmonella* isolation

Figure 4: Cecum content collection in slaughter house



A B

Figure 5 A) RVS medium before inoculation B) RVS after inoculation with samples from pre-enrichment medium with evidence of growth of Salmonella.



A B

Figure 6: A) Salmonella colony on S-S agar B) Salmonella colony on XLD agar

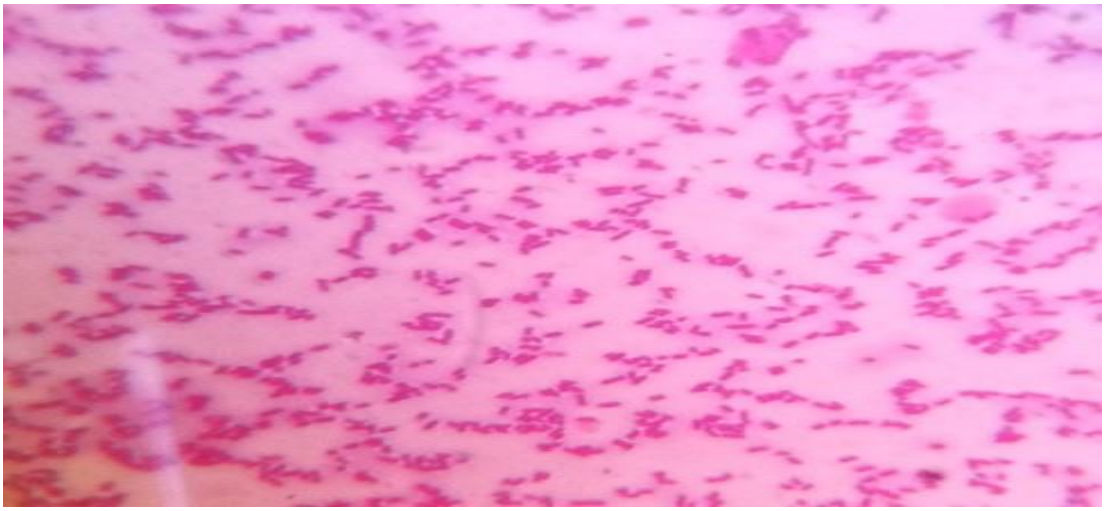


Figure 7: Microscopic examination of stained smear from suspected colonies of Salmonella on XLD agar

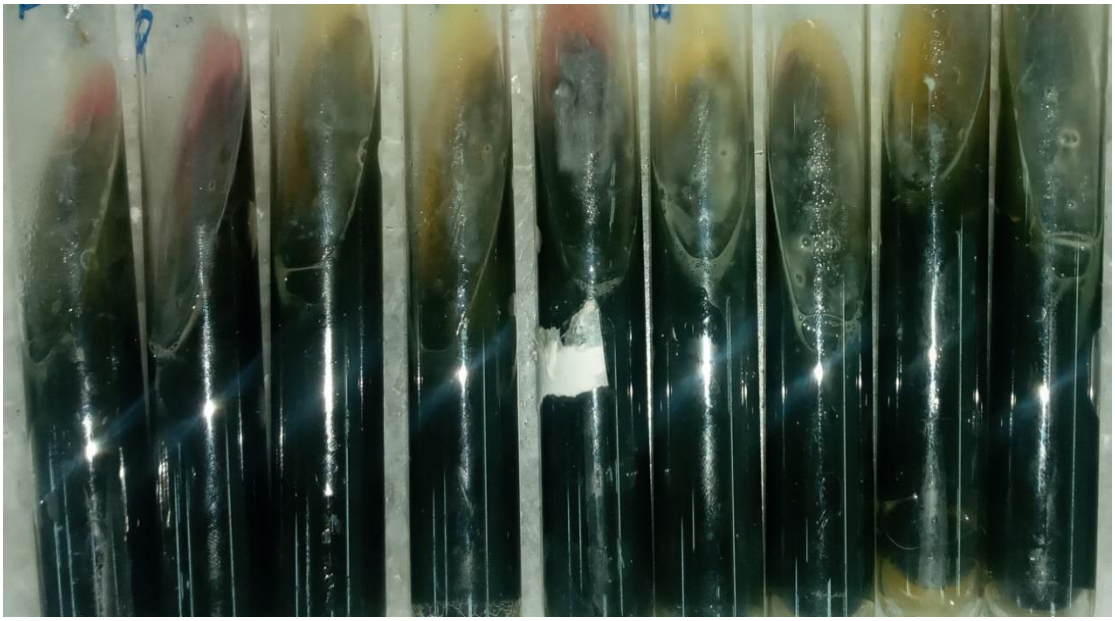


Figure 8: Results of TSI test on suspected colonies of Salmonella: Black color indicates production of H2S by Salmonella organism.

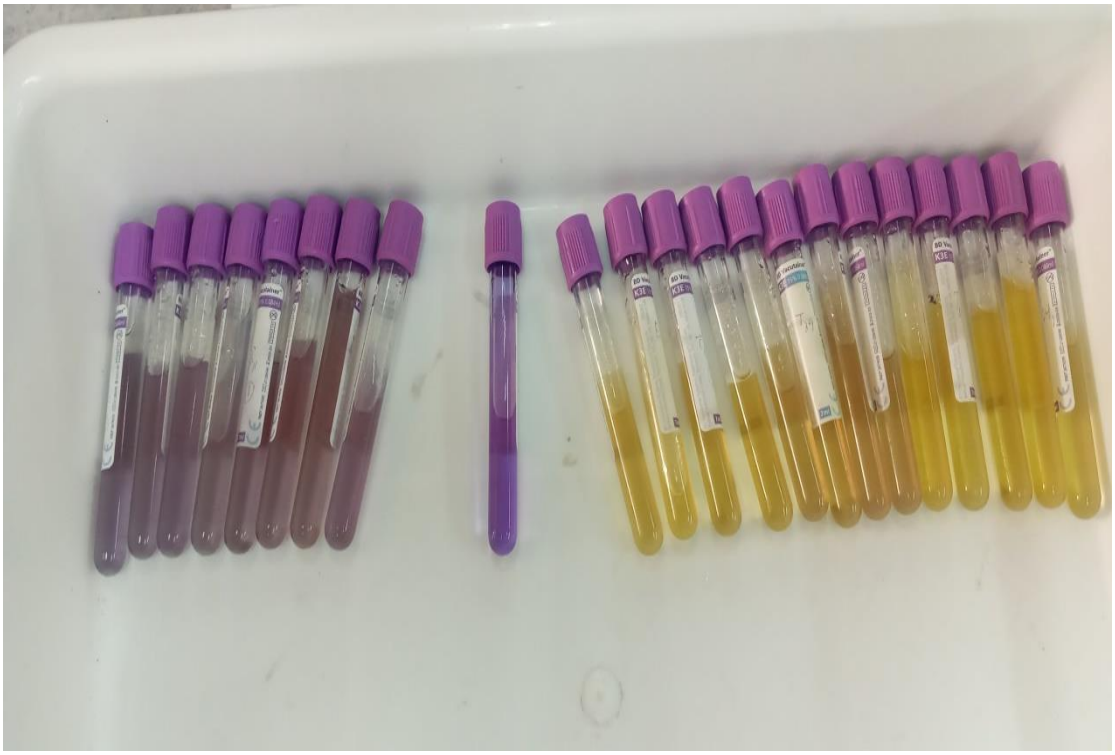


Figure 9: Characteristics Salmonella on Lysine decarboxylase broth: left Salmonella typhi; right Salmonella paratyphi

Figure 10: Growth characteristics of suspected Salmonella on Simon citrate agar

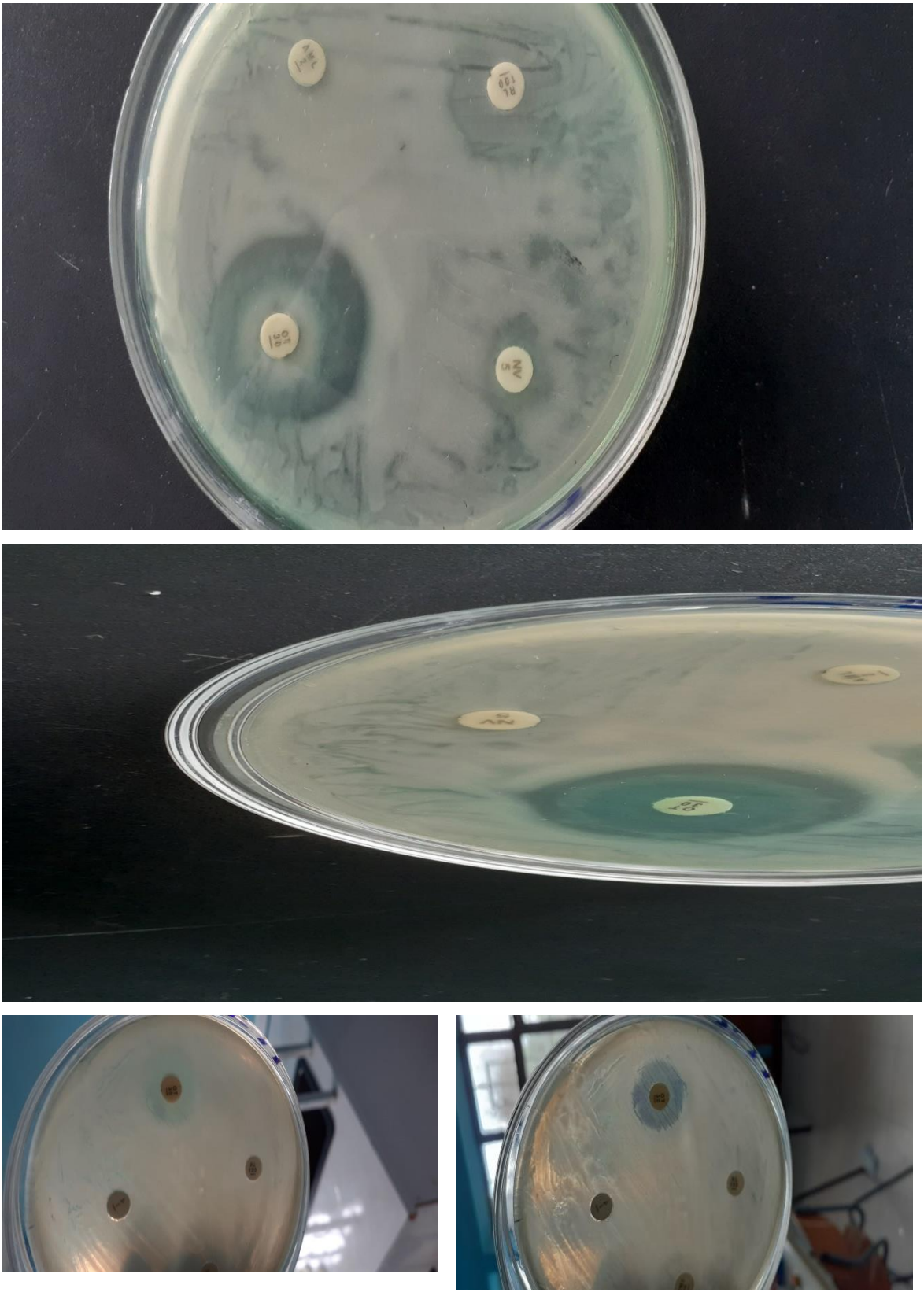


Figure 11: Antimicrobial susceptibility test

Annex 8 : Ethical clearance form approved by Ethics Review Committee of CVMA

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/16/05/13/2021

Name of Applicant: **Tesfaye Belachew (DVM, MVSc fellow)**

Address: Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project*: Isolation and identification of Salmonella species from small holder broiler chicken farms and their antibiograms in Central Ethiopia: implication for public health*

Date of application:	**February, 2021**
Nature of the project:	**Mildly invasive**
Target animal species:	**Domestic chicken**
Number of animals involved:	**389**
Study area:	**Central Ethiopia**

Minutes No. and date of review: **VM/ERC/05/13/021, 21/03/2021**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when deemed necessary

Getachew Terefe (DVM, PhD)
Chairman

Signature

The Federal Democratic Republic of Ethiopia
Addis Ababa University
College of Veterinary Medicine and Agriculture

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Please quote Our Ref. No. When replying

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Fax 251-11-4339933 Tel. +251 114338450 P.o.x. Box}34 Bishoftu, Ethiopia