Thesis Ref No._____

APPRAISAL OF BIOSECURITY AND OCCURRENCE OF *SALMONELLA* IN SELECTED SMALL AND MEDIUM SCALE CHICKEN FARMS AT ADDIS ABABA, ETHIOPIA

MSc THESIS

BY

TSEDAL MULUNEH AYELE

ADDIS ABABA UNVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE
DEPARTMENT OF ANIMAL PRODUCTION STUDIES

AUGUST 2021

BISHOFTU, ETHIOPIA

APPRAISAL OF BIOSECURITY AND OCCURRENCE OF *SALMONELLA* IN SELECTED SMALL SCALE AND MEDIUM SCALE CHICKEN FARMS AT ADDIS ABABA, ETHIOPIA

Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Animal Production

By:

Tsedal Muluneh Ayele

AUGUST2021

BISHOFTU, ETHIOPIA

SIGNATURE SHEET

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Animal Production Studies

As MSc research advisors, we here by certify that we have read and evaluated this Thesis prepared under our guidance by Tsedal Muluneh, entitled "**APPRAISAL OF BIOSECURITY AND OCCURRENCE OF *SALMONELLA* IN SELECTED SMALL AND MEDIUM SCALE CHICKEN FARMS AT ADDIS ABABA, ETHIOPIA**"

Submitted by: Tsedal Muluneh Ayele _____ _____

Name of Student Signature Date

Approved for submittal to a thesis assessment committee:

1. Gebreyohannes Berhane (PhD, Assoc. Professor) _____ _____

Major Advisor Signature Date

2. Hika Waktole (Assist. Professor) _____ _____

Co-Advisor Signature Date

3. Tadesse Eguale (PhD, Assoc. Professor) _____ _____

Co-Advisor Signature Date

4. Gebreyohannes Berhane (PhD, Assoc. professor) _____ _____

Department Chairperson Signature Date

APPROVAL SHEET

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Animal Production 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: Tsedal Muluneh. Entitled "**Appraisal of Biosecurity and Occurrence of *Salmonella* in Selected Small and Medium Scale Chicken Farms at Addis Ababa, Ethiopia**" and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Animal Production.

Prof. Yacob Hailu	_____	_____
Chairman	Signature	Date
Dr. Ashenafi Mengistu	_____	_____
Internal Examiner	Signature	Date
Prof. Adem Hiko	_____	_____
External Examiner	Signature	Date

DEDICATIONS

This thesis is dedicated to my beloved mother, Alem Tadesse, and my best and most indestructible father, Muluneh Ayele. You have raised me with love and made many sacrifices to be able to owe me at this stage.

STATEMENT OF THE AUTHOR

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

Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

Name: Tsedal Muluneh Ayele Signature: _____

Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: _____

ACKNOWLEDGEMENTS

First and for most, I would like to thank the almighty of God and also I am great full for my families who owe me at this stage.

Let me use this prospect to acknowledge Addis Ababa University for providing me this MSc. Program through Female's Scholarships. I have gain fully funded opportunity that lets me to gain intended financial supports.

I wish to forward my heart left gratitude for Hika Waktole (Assistant Professor) because he was the one who compassionate and optimistic to comprise me in this ongoing project, which allows me to achieve financial and material supports and also for his valuable advice.

Next, my special thanks go to my advisor Dr. Gebreyohannes Berhane (PhD, Associate Professor), He has helped me not just by giving a research assistantship, but also by being empathetic and encouraging since the beginning of my research activities and along rough road to finish this thesis. I am also grateful for Prof. Kebede Amenu (PhD), his enthusiasm to share knowledge was astonishing. I have gained vast experiences those help me in chasing research career in present and future.

By using this opportunity, I would like to acknowledge Aklilu Lemma Institute of Pathobiology for the accommodations of accessing their Micro-biology Laboratory and my special thanks goes to Dr. Tadesse Eguale (Associate Professor) for his interminable support; humbleness, endless patience, massive consultations and critiques that help me in elevate my scientific knowledge. I am also thank full for Haile Alemayehu (Assistant professor), his boundless and cordial supervisions makes me understood a lot in Microbiology.

Finally, Sub-City level Agricultural officers deserve to be accredited. It would be very hard to visit the farms without their assistance.

TABLE OF CONTENTS

LIST OF TABLES

Characteristics of explored chicken farms

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

BPW	Buffer Peptone Water
DNA	Deoxyribonucleic acid
FAO	Food and Agricultural Organization
H_2S	Hydrogen Sulphide
HACCP	Hazard analysis and critical control points
ISO	International Organization for Standardization
LIA	Lysine Iron Agar
SIM	Sulfide Indole Motility
TSI	Triple Sugar Iron agar
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

ABSTRACT

Biosecurity is believed to have the ability to improve production and ensuring the safety of chicken products through minimizing pathogenic infections such as salmonellosis in poultry. The aim of this study was to assess biosecurity measures and investigate the occurrence of *Salmonella* in small and medium scale poultry farms at Addis Ababa, Ethiopia. A cross-sectional study was conducted to collect data and poultry house related samples. A total of 56 farms were addressed through the questionnaire survey by using KoBoCollec data collection tool and again a total of 391 biological samples (223 fecal droppings, 56 drinking water, 56 feed, and 56, floor swabs) were collected and analyzed for *Salmonella* Spp. following standard laboratory techniques. The questionnaire responses data were analyzed through descriptive data analysis and then biosecurity scoring system was developed to result ten basic biosecurity components. Multiple response analysis (MRA) was conducted to determine adoption level of each biosecurity components above mean score ("good") across total assessed farms. The results showed that biosecurity in feed and water management and also in infrastructure of the farms were implemented in 87.5% and 76.8% respectively, while farm entry restrictions and farm relative location were slacked (16.1% and 3.6%, respectively). The adoption level of disease management practices were 64.3% and 48.3% of farms implemented cleaning and disinfection practices above mean score. *Salmonella* was identified in 15 (26.8%) of the farms and 22 (5.6%) of the samples. Occurrence of *Salmonella* was higher in small scale poultry farms (21.4%), deep litter farms (21.4%), farms containing layers (25%) and all from Bovans brown breeds. Farms with score of "Bad" were found to exhibit high number *Salmonella* comparing to farms implement biosecurity components as good. This signposted the benefits of applying biosecurity measures in poultry production to eliminate consequences of production loss and zoonosis due to bacterial infections such as salmonellosis.

Key Words: *Biosecurity Score, Zoonotic disease, Salmonella, Chicken farm*

1. INTRODUCTION

Ethiopia established and determined master plan which reliefs the interest of increasing chicken meat and egg production from 2015-2020. Responsible livestock centers (Poultry breeding, multiplication and dissemination centers) in the country have mandated with importing, intensifying and distributing commercial chickens to end users since the poultry production is considered to be an essential source of domestic food and nutritional security in the country. Conversely, the sector is facing several hindrances comprising infectious and parasitic diseases because the biosecurity and the pathogenic patterns of the centers have not been estimated as to whether the centers performing accordingly with their task effectively without any risk (Asfaw *et al*., 2017 ; Abdi *et al.*, 2017).

The concept of biosecurity is principally related to the prevention of different pathogens from infesting farms and farm environments, and also the elimination of previously existing pathogens from spreading through sanitary and hygienic measures in the broad sense (Andres and Davies, 2015). External biosecurity in the manner of purchase of chickens, removal of manure and dead chickens, restriction of vehicles and visitors, infrastructure and biological vector control, and internal components of biosecurity such as management of disease, cleaning and disinfection, materials and measures between compartments are salubrious for the purpose of reducing animal health complications and also reducing the use of antibiotics, which are substantial for advancement of animal products and production (Cuc*et al*., 2020; Gelaude *et al*., 2014).

Particular control measures of biosecurity at farm level are directly related to prevalence of infectious diseases caused by bacteria such as *Salmonella* with cross-contaminations (Asfaw *et al.*, 2017). Salmonellosis is one of frequently reported zoonotic disease, generating global public health concern. The disease caused by different types of *Salmonella* serotypes which are aerobic and facultative anaerobic and gram-negative rods morphologically. The genus *Salmonella* consists of only two species, *Salmonella bongori* and *Salmonella enterica*, with the latter being divided

into six sub species: *S. enterica* subsp. *enterica, S. enterica* subsp. *salamae, S. enteric* subsp. *arizonae, S. enterica* subsp. *diarizonae, S. enterica* subsp.*S. houtenae*, and *S. enterica*subsp. *indic* (Lamas *et al*., 2018).

Now a days, consumption of meat products including contaminated poultry meat is considered to be main source of human infection with *Salmonella* (Antunes *et al*., 2016). FAO, (2019) lineup salmonellosis with major poultry diseases which have consequential role in Ethiopian poultry production along with Newcastle disease, Infectious bursal disease, Marek's disease, Mycoplasmosis, Colibasilosis, Coccidiosis, Toxoplasmosis and Heliminthosis.

Biosecurity is believed to be important practice in ensuring the health and productivity of food producing animals at different levels such as private and governmental enterprises, regions and country. This can be achieved when the disciplines, obligation, and guidelines of biosecurity are enforced to prevent the introduction and spread of diseases (Robertson, 2020). A study was conducted by Soliman and Abdallah (2020) to assess the intensity of biosecurity measures for the occurrence of *Salmonella*. According to the author's conclusion, weak biosecurity measures in poultry houses (opened and closed) have multifarious influence on route of entry and multiplication of *Salmonella* species.

Animal production with a low level of biosecurity practices plays a crucial role in different public health zoonotic and economic risks. Millions of cases are reported each year around the world due to pathogenic bacteria such as *Salmonella* and Most of the cases are related to animals and animal products (WHO, 2015). Poor biosecurity also affect animal production sector since it has consequences of production loses such as low production from expected and also animal mortality (Wong *et al*., 2017).

Biosecurity status on chicken farms demands strengthening of the existing measures through training and awareness creation and to the farm owners and their employees since inadequate biosecurity status is observed on small and medium scale chicken farms in Ethiopia (Ismael *et al.*, 2021 ; Haftom *et al.*, 2015).

Even while the concept of biosecurity is known to be a vital part of safe and profitable poultry production, the attention given is incomparable to that of breeding and nutrition regimes and many studies have been launched on the prevalence of different pathogenic bacteria, including *Salmonella* in Ethiopia. On this behalf, the objective of this study was:

General Objective

To assess biosecurity measures in selected small and medium-scale chicken farms at Addis Ababa linked to occurrences of pathogens as reference level of biosecurity, with special interest in *Salmonella*.

Specific objective

- ✓ Exploring implementation of different biosecurity prospectuses in small and medium poultry farms in Addis Ababa city ;
- ✓ Isolation and identification of *Salmonella* from poultry and poultry related samples to estimate occurrence of *Salmonella* ; and
- ✓ Determining association between biosecurity components adoption level and occurrence of *Salmonella*.

2. LITERATURE REVIEW

2.1. Explanation of Poultry Farm Biosecurity

Can (2018) defines biosecurity as "comprehensive infection management practices that take into account education level, sociocultural characteristics and perceptions, geographical, climatic, and epidemiological circumstances." In addition, biosecurity is the most effective way to avoid further potential losses in the future. Increased training and specialization, improved connection with professional groups and institutions, and a more holistic approach to the biosecurity concept will all help to tackle existing issues. To put it another way, producers should analyze the features of their own businesses and calculate the appropriate mix of biosecurity measures.

The high susceptibility of poultry to disease outbreaks makes a comprehensive biosecurity technology a necessary practice in poultry farms to protect the farms from both intentional and unintentional threats from biological agents (Abdelgadir and Ismail, 2017). Maduka *et al*. (2016) did a study on on-farm biosecurity methods at commercial chicken farms in Jos, Nigeria. According to the authors, the chicken production system requires a greater commitment to excellent biosecurity measures, and biosecurity flaws might be addressed by providing farmers with more information in order to assist the expansion of chicken production with robust biosecurity measures that drastically reduce risk of disease outbreak.

For healthy animal production, good biosecurity precautions are essential. In chicken farms, the absence or disregard of biosecurity practices can result in unusual conditions such as a high mortality rate, reduced profit, and investment loss. Infection of layers with S. enterica *serovar gallinarum* can cause significant reduction in egg production and weight loss. Therefore, it is preferable to adhere strict biosecurity measures as means of prevention and control of fowl typhoid in poultry farms, as this disease could lead to decrease in egg production, weight loss and other eggshell abnormalities (Tasie*et al.*, 2020 ; Limbergen *et al*., 2018; Chiroma *et al.*, 2018).

2.2.External Biosecurity

2.2.1. Chicken purchasing practices

To ensure a homogenous population of birds at the broiler farm, it is essential to populate all chicken houses with those of the same hatchery and breeder farm because purchasing animals from various farms increases the danger of disease-causing organisms being introduced (Gelaude*et al*., 2014). Biosecurity measures related to transport vehicles or trucks is also related to risk of spreading disease. Additionally, using of disinfected delivery trucks, delivery of chickens to only one farm at a time and delivery from same source is recommended to reduce those risks related to chicken purchasing practices (Tanquilut *et al*., 2018).

2.2.2. Feeding and watering management

According to Zewdu and Wondimagegn (2017), poultry feed can get contaminated with various bacteria during harvesting, processing at feed mill, or storage, as well as through dust in feed mills. Controlling dust, regulating personnel flow, reducing fat buildup, controlling rats and wild birds, and maintaining the hygiene of transport vehicles are the most essential preventative methods to be used for feed contamination. Concurrently, protecting water sources from vermin and other animals and basic biosecurity (chlorinated mains water, covered header tanks, cleaning and disinfection of tanks, and lines between flocks) seems to maintain these low risk levels since contaminated feed and water can result in the introduction of diseases (Robertson, 2020).

2.2.3. Farm entry restrictions

Controlling human and vehicle traffic-flow into the farm, as well as within and outside the farm's premises, are essential for preventing disease spread and ensuring effective biosecurity. Biosecurity techniques including poultry farm fencing, human and vehicle control within and into the farm are referred to as traffic flow control. Visitors should not come into direct contact with the birds, and material, supplies, equipment, finished final products, movement should be controlled (Fathelrahman *et*

al., 2020). The potential movement of staff between sheds posed the highest risk to layer and broiler operations, while the on and off-farm movement of commodities and services posed the highest danger to breeder operations (Greening *et al.*, 2020).

2.2.4. Relative location of farm, infrastructure and control of biological vectors

The geographical situation of poultry farms is likely to have an influence over the biosecurity practices to be implemented. In general terms, the higher the density of poultry farms in the area, the greater the risk of introducing disease and the stricter the biosecurity measures that have to be applied to avoid the spread of infections between herds (Andres and Davies, 2015). In case of increasing the distance among farms is not possible, it is recommended to install fences, which effectively prevent people and other domestic animals from entering the poultry operation without control. Vegetation could also improve the barrier surrounding the farms. Among other sources of risk, in addition to the proximity of the farms, are highways and vicinal roads, which allow free access to the farms (Hergot *et al*., 2021).

The role of carrier vectors in the transmission of *Salmonella* and other organisms is widely accepted and well-discussed. Rodents, birds, insects, feral animals, dogs, and cats can all potentially mechanically transmit pathogens. Among them, rodents are of particular importance. While the popular belief is that pests act as a source of introduction of the disease in the herd, it is more likely that they act as a reservoir of farm-resident strains, recycling the infection from one crop to the next (Andres and Davies, 2015).

Carrier vectors have high capacity to carry different salmonella species. A single swab of wild bird feces collected from outside the shed housing Flock A was positive for S. Typhimurium and Mites harvested from birds infected with serovar Gallinarum were shown to carry the mite, and then to transfer serovar Gallinarum to isolated groups of pathogen-free birds (Mcwhorter and Chousalkar, 2019 ; Geer *et al*., 2020).

2.3. Internal Biosecurity

2.3.1. Cleaning, disinfection and sanitation measures

Poultry farming faces a major environmental challenge associated with waste generation, its adequate treatment and disposal. As a result, methods considering both the environmental impact and safe waste disposal should be prioritized (Laroucau *et al*., 2020).

Control of photogenic pattern in poultry farms regardless of the housing system, location, holding capacity, and ventilation system depend mainly on strict measures including good hygienic measures, early detection, and effective cleaning and disinfection program. Cleaning and disinfection programs are observed to ensure applying biosecurity measures announced by different farm. Dry cleaning can be applied to remove loose dirt, followed by wet cleaning using detergent and water under pressure, with paying attention for corners, joints, and fissures in walls and floors. Most predominating detergents includes quaternary ammonium compounds or a mixture of aldehydes and quaternary ammonium compounds (Soliman and Abdallah, 2020).

Cleaning reduced the bacterial load, the first disinfection step using either peracetic acid or fogging with formaldehyde reduced the *Salmonella* further but did not eliminate it, with the air supply systems remaining positive. The inclusion of a second disinfection step using sodium hypochlorite and external application of lime eliminated *Salmonella* on one farm completely and within the internal buildings of the second, although it did persist in low levels in the external environment (Gosling, 2018).

Pulido-landínez, (2019) explored the sources of *Salmonella* in broilers which needs an intervention to reduce horizontal transmission, dissemination and persistence of *Salmonella*. *Salmonella* free baby chicken placed in a *Salmonella* free chicken house environment, microbiological, physical, and chemical water characteristics, pest control and effective rodents control, control of *Salmonella* carriers wild birds

(especially pigeons), prevent presence of *Alphitobius diaperinus*, flies, and mites (*Dermanyssus gallinae*) it is necessary to decrease horizontal transmission, *Salmonella* survival in dead birds since it is able to survive in the liver up to 148 days at −20 °C and could also be in bone marrow for up to 90 days, Drinker management and feed managements to condense letter moisture by drinkers and diminish contamination of feed by feces are major prevention and control methods those mentioned by the study (Hergot *et al.*, 2021)

2.3.2. Management of sick and dead birds

The chicken production system requires a greater motivation for excellent biosecurity practices, and biosecurity weaknesses could be addressed by providing farmers with information in order to support the expansion of chicken production with robust biosecurity measures that drastically reduce the risk of disease outbreaks (Maduka *et al*., 2016). Confinement of animals within a controlled environment is essential to prevent risk and to keep high biosecurity standards. In general, isolation aims to minimize any potential source of contamination from the farm, so isolation applies to control entering and exiting the farm (Fathelrahman *et al.*, 2020). Survey analysis by Haftom *et al.* (2015) shows that all in all out practice, keeping different age groups together and no quarantine of new comer birds and disease birds were major consequences in their study area.

Burning of dead birds as a disposal method limits the susceptibility of broilers to infectious diseases. Presence of quarantine area in the broiler house limits the spread of diseases from sick birds to other healthy birds and also birds earlier sold and rejected by consumers, are put under quarantine for a period of at least three weeks limiting susceptibility of resident broilers to disease (Oluwasusi *et al*., 2018).

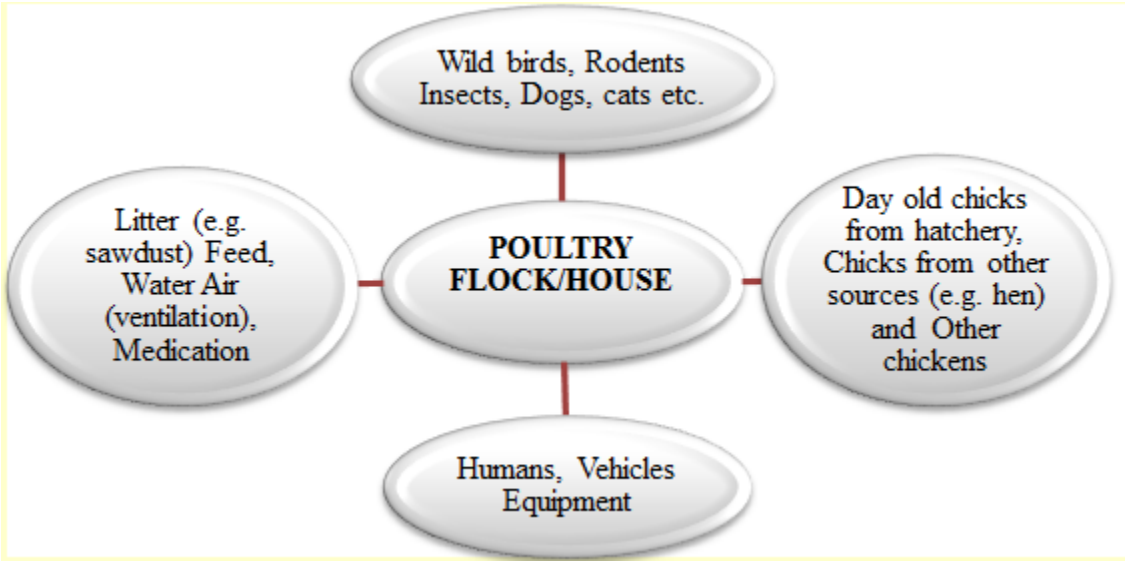


Figure 1: Possible Source of contaminants for poultry houses, Adapted from : Aila, (2012)

2.4. Recommended Biosecurity Measures for Small and Medium Scale Poultry Farms

As synopsis by Conan *et al*.,(2012) on biosecurity measures for poultry in developing countries, biosecurity is indispensable tool to mitigate spread of infectious disease even though the recommendations are not applicable and also it is difficult to achieve general guide line related to poultry farm biosecurity. Yitbarek and Mengistu, (2016) also indicate that almost all the poultry producers did not know the type of disease that occurred in their farms and were not accustomed to recording the disease that occurred.

The above statement was supported by Haggag *et al*., (2018) since there result shows small scale poultry farms with low biosecurity measures are associated with infectious diseases. According to their outcome there was a significant association between the application of traffic control measures and the prevalence of avian influenza. Also, it was noticed that there was a significant association between the application of sanitation measures and the prevalence of this disease and prevalence was higher in broiler houses showed relaxed biosecurity measures compared to other houses with strict biosecurity measures.

Abdelgadir and Ismail (2017) indicated biosecurity pointers which require attention in small and medium scale intensive farms. location and distance to nearest farms, level of biosecurity at farm gate, level of biosecurity between the farm gate and the shed, biosecurity measures related to isolation, water sanitation and water system cleaning and source of feeding and protection feed stores are considered to be major focusing areas when best biosecurity activities are taken into consideration in small and medium scale poultry farms.

2.5. Zoonotic and Economic Importance of *Salmonella* in Ethiopia

Salmonella is bacteria that live in the intestinal tract of carrier animals of many species including livestock, poultry and reptiles. Infective numbers of the bacteria are shed into the faces of these animals particularly during periods of stress such as being yarded and transported. Other animals and humans can get the *Salmonella* through direct or indirect contact with fecal material and infection then produces gastroenteritis (Morwal and Sk, 2017). Hence *Salmonella*e are ubiquitous, found in animals, humans, and the environment, a condition which facilitates transmission and cross contamination also exert huge health and economic impacts due to their virulence or carriage of antibiotic resistance traits. To address this significant issues with regard to public health, availability of adequate information on the prevalence and antibiotic resistance patterns of *Salmonella*, and establishment of adequate measures to control contamination and infection are needed (Kebede *et al*., 2016).

Salmonella is one of zoonotic and socioeconomically important bacterium in developing countries including Ethiopia. Most of the time *Salmonella* outbreaks are arises from poultry and poultry products and ingestion of huge amount *Salmonella* will bring gastroenteritis. Since human cases of salmonellosis are being increased, application of preventive methods either at farm level or processing level will be beneficial.(Zewdu and Wondimagegn, 2017)

Salmonella isolation from poultry farms by Jaleta *et al.* (2017) showed the distribution is not limited to sample type, poultry breeds, age and chicken production stage indicating its widespread and ubiquitous nature. Most of isolates were motile

that reflects the majority of isolates have probability of zoonotic potential. Alarmingly, majority of the isolates have developed multi-drug resistance endangering poultry production and public health as these drugs are used widely for treatment and prophylaxis in animals and humans.

2.6.Occurrence of Salmonellosis and Biosecurity Measures in Ethiopia

Salmonella spices have high prevalence in different regions of Ethiopia including Jimma town, Debrezyiet and Addis Ababa from poultry products such as chicken carcass, giblets, eggshell and egg content (Abebe *et al*., 2020). Studies conducted related to biosecurity practices in other parts of the country including Mekelle, around Modjo and Debremarkos indicated that biosecurity practices such as all in all out practice, categorizing same age and sex together, absence of quarantine of new coning birds, inappropriate disposal of dead bird and inadequate chemical application for the sake of sanitary purpose are not well practiced (Haftom *et al*., 2015 ;Yitbarek and Mengistu, 2016 ; Jaleta *et al*., 2016).

The lack of commitment to the adopted measures contributed a breach through which microorganisms can enter, stabilize themselves, develop infectious and contagious diseases, and spread from one area to another inside the same farm or to other farms easily. Control of salmonellosis in broiler farms regardless of the housing system, location, holding capacity, and ventilation system depend mainly on strict measures including good hygienic measures, early detection, and effective cleaning and disinfection program (Soliman and Abdallah, 2020).

Poultry breeding, multiplication and distribution centers in Ethiopia, as they currently stand, are a source for the dissemination of pathogens and drug resistant pathogens, at least *Salmonella*. *Salmonella* was recovered from chicken cloaca, the environment (bedding) and human hands in the multiplication centers. Its distribution was associated with location of the individual center/farm, age of the chickens and the sample type indicating these factors are important potential factors for intervention. Substandard management practices and poor biosecurity exposes the birds to numerous potential sources of *Salmonella* (Abdi *et al.*, 2017).

Biosecurity measures were evaluated by Abdelgadir and Ismail, (2017) and their study shows that unfitting biosecurity routines leads to appearance of *Salmonella* spices to layers farm. Accordingly, absence of routine pest control, access points for rodents and wild animals are major problem related to *Salmonella* infections since they are considered as victors. The other important concept is record keeping, which is very essential when early assistance of poultry health issue and response to any biosecurity breach is taken into consideration.

2.7 Control and Prevention of Salmonellosis through Biosecurity Measures

Disease control and prevention require a multifaceted method with a thorough knowledge of the current disease situation in an enterprise, the likely disease threats, and how the risk of introduction can be minimized. Such an approach requires a sound knowledge of the discipline of Veterinary Epidemiology, with an understanding of disease transmission and spread, risk factors for disease, and methods to prevent disease (Robertson, 2020).

Meat, dairy products, and eggs are the main ways by which people are exposed to zoonotic bacteria. *S. aureus, Salmonella species, Campylobacter species, L. monocytogenes,* and *E. coli* are the major zoonotic bacterial pathogens which are the causative agents of food-borne illness and death in the world associated with consumption of contaminated animal products. Good hygiene, sanitation in operating procedures, and implementation of standardized Hazard analysis and critical control point (HACCP) and pasteurization procedures are effective methods for the control and prevention. Currently, the emergence of multidrug-resistant zoonotic bacteria associated with consumption of contaminated animal products is a great concern for the public health, and there should be coordinated surveillance and monitoring system for food-borne zoonotic bacterial pathogens particularly in developing countries including Ethiopia (Abebe *et al*., 2020).

Different biosecurity based options those help to avoid *Salmonella* infestation are indicated by Zewdu and Wondimagegn (2017) which comprises flock management (litter, dust, mice, flies and different surfaces) withdrawal of feed for molting purpose,

feed mill or storage management, flock testing, sanitation, vaccination, electrolyzed water, and micro wave technologies. Instituting biosecurity and bio-containment practices in addition to enhanced food processing method and preparation and storage practices are required to control and prevent food spoilage due to *Salmonella*. Attenuated DNA recombinant live *Salmonella* vaccines combined with comprehensive control strategy in animals, feed, and animal foodstuff will help to reduce salmonellosis (Abebe *et al*., 2020).

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Addis Ababa, the capital city of Ethiopia on small and medium scale poultry farms. Addis Ababa is located in the center of the country on the edge of the central plateau. The city lays at $9^0$1'48" N latitudes and 38^0 44'24" E longitudes. It has an altitude of 2100 meters in the southern city to 3000 meters above sea level in the northern part of the city (NMSA, 2002). Annual rain fall of the city is 1800 mm and annual temperature varies between 11°C and 26 °C. Total population of chicken in Addis Ababa city is about 100,163 from 25,616 households which comprise 0.23% of countries total chicken population size (FAO, 2019). Map of study area and specific locations of poultry farms involved in the study is shown in **Figure 2**.

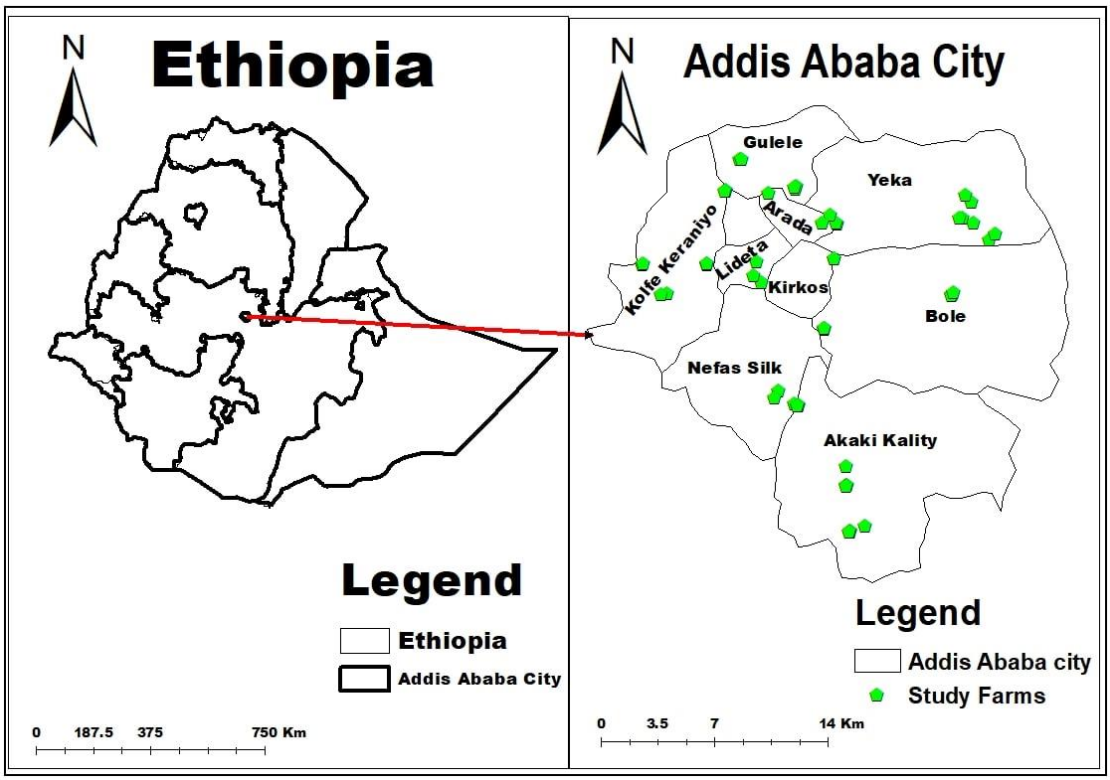


Figure 2: Map of the study area

Study Design

Assessment of biosecurity implementations on small and medium scale chicken farms and investigation of *Salmonella* was conducted through cross-sectional study design between October 2020 and May 2021.

List of registered farms (n=104) were obtained from farmers and Urban Agriculture Development Commission of Addis Ababa, Farmers' Extension Directorate and farms were included to the study purposively based on their availability, owner's willingness and according to study's inclusion criteria. Finally, total of 56 poultry farms were indicated by sub-city level professionals (urban agriculture officers) from ten sub-cites of Addis Ababa city.

3.2 Study Population

Small-scale intensive and semi-intensive chicken production systems are found both in urban and rural areas of Ethiopia. However, the former is mainly concentrated in urban and peri-urban areas in the central parts of the country (FAO, 2019). In current study total of 56 (42 Small scale with 100-1000 birds and 14 medium scale with 1001-5000 birds) poultry farms were indicated by sub-city level professionals (urban agriculture officers) from ten sub-cites of Addis Ababa city (Eguale *et al*., 2016) Farms with stock size ≥ 100, Chickens at all age and sex and apparently healthy chickens were included in the study. Eligible participants of biosecurity assessment were farm owners, farm managers and other responsible individuals from selected farms.

Table 1: Characteristics of explored chicken farms

Biosecurity measures	Responses	Frequency	P (%)
Purpose of chicken farming	Layers	50	89.3
	Broiler	6	10.7
Keeping different type of breeds	Yes*	1	1.8
Age of chickens	2-6 months	15	26.7
	7-12 months	25	44.6
	>12 months	16	28.5
Housing system	Deep litter system	47	83.9
	Cage system	7	12.5
	Mixed	2	3.6
Breeds of chickens	Bovans brown	50	89.3
	Sasso	5	8.9
	Mixed	1	1.8
Stock size of farm	Small scale	42	75
	Medium scale	14	25
All in all out practice	Yes*	44	78.6
Rearing of other animal spices	Yes *	18	32.1

3.3. Questionnaire Survey

3.3.1 Questionnaire construction and deployment

With the intention of functionally characterize essential biosecurity parameters, most of the questions were adopted from ***Biocheck.UGent™*** tool (https://biocheck.ugent.be/en), scientific scoring system to evaluate quality of on-farm biosecurity for poultry, pigs, and cattle which have been validated by recent scientific literatures in using this assessment tool (Limbergen *et al.*, 2017 ; Tanquilut *et al.*, 2018 ; Caekebeke *et al.*, 2020). Then after, questions were modified with relevance to poultry production system in Ethiopia and along with the specific study site.

Questionnaires comprised both external and internal biosecurity parameters with sub-sections of general characteristics of farms and respondents, chicken farm characteristics, external biosecurity (chicken purchasing practices, feeding and watering, manure and other waste management, farm entry restriction and material sharing, farm infrastructure and biological factors) and internal biosecurity (poultry disease management and cleaning, disinfection and sanitation measures).

KoBoToolbox was used for data collection by importing the questions to online platform and android- based data collection software (***KoBoCollect,*** version 1.30) was used finally for data collection (Pham *et al*., 2019) (**Appendix I**).

Table 2: characteristics of study participants (n= 56)

Variables	Categories	N	P (%)
Role of respondent	Farm owner/co-owner	46	82.1
	Employee	9	16.1
	Owners relative	1	1.8
Sex	Male	34	60.7
	Female	22	39
Age	18-25	1	1.8
	26-35	29	51.8
	36-45	20	35.7
	>45	6	10.7
Educational Status	No formal education	4	7.1
	Primary school	15	26.85
	Secondary school	24	42.9
	Diploma and above	13	23.2
Service year	<5	48	85.7
	5-10	8	14.3
	≥10	0	0
Marital status	Married	27	48.2
	Unmarried	20	35.7
	Divorced	7	12.5
	Widowed	2	3.6

3.3.2. Data collection

The organized questionnaire was piloted prior to assessing the selected farms on 5 non-participating farms for better clarity of questions and acceptability of the data collecting tool on the field. Following the pilot test, some of the questions were modified and some options were deleted or included.

At the time of data collection, the objective of the study were clearly explained for the eligible participants of biosecurity assessment that include farm owners, farm managers or other responsible individuals from selected farms also written consents were acquired. The actual date and time, before administering the major section of the evaluation, Geographical Point of the farms, general character of the farm and respondents were all recorded. At the end, the finalized form for each farm was uploaded to the project created on KoBoToolbox server.

3.4 Bacteriological Investigation of *Salmonella*

3.4.1 Sample Size Determination and Sampling Technique

Number of collected fecal dropping samples were estimated according to Thrusfield, (2007) with 95 % confidence interval, ±5 % of allowable error and the expected prevalence of 16.5% Eguale, (2018). Initially 212 samples were estimated however, 5% of estimated sample size was added with the intention of increasing the precision to result 223 total collected fecal droppings. The estimated sample size was allocated through proportional random allocation based on the flock size of the farms and the remaining environmental samples such as water samples, feed samples and floor swabs were collected per farm level (56 each). On this base, the overall total sample size was 391.

$$\underline{n= (z^2)\ p\ (1-p)} \quad \text{where,}$$
$$d^2$$

n=sample size

Z= z statistic for the level of confidence (1.96 usually if we use 95% of confidence of interval)

P= expected prevalence

d= allowable error/desired absolute precision

3.4.2 Sample Collection and Transportation

Entirely 391 samples (223 of pooled fresh fecal droppings (3-chicken each), 56 feed samples, 56 water samples and 56 floor swabs) were collected and investigated. Minimum of 5g fecal droppings and feed samples were collected by using clean disposable gloves in to sterile zippered plastic bags while 20 ml water samples were obtained from 5 different drinkers (4ml from each) at a given farm using sterile falcon tubes. The floor swab samples were collected by swabbing the floor with BPW moisturized cotton swabs (10 cm x 10cm) from two opposite directions of the floor and pooled into falcon tube containing 45ml of BPW. Ultimately, all samples were transported within 3–4 h of collection to Microbiology Laboratory of Aklilu Lemma Institute of Pathobiology, Addis Ababa University using an icebox containing ice pack.

Table 3: Summary of total collected specimens

Sample type	**Number of samples collected**
Pooled fecal droppings	223
Water	56
Feed	56
Floor swab	56
Total	***391***

3.4.3 Isolation and Identification of Salmonella

Salmonella isolation and identification was conducted according to the technique/procedures recommended by International Organization for Standardization ISO-6579, (2002) and WHO, (2010) global Food- borne Infections Network Laboratory Protocol, Isolation of *Salmonella* Spp From Food and Animal Feaces, inclusive of pre-enrichment, selective enrichment, Plating onto selective and

differential media. Biochemical confirmation of *Salmonella* isolates were attempted through Triple Sugar Iron tests, Simmons citrate, lysine decarboxylase, sulfur Indole production Motility Test (SIM) and Urease tests.

Pre- enrichment and selective enrichment

For the purposes of isolation and identification of *Salmonella*, pooled samples of 5g fecal dropping and 5g of feed sample were pre- enriched into falcon tubes containing 45ml of BPW (OXOID England) after homogenization there by incubated for 24hr at 37^{o}C. Likewise, 20 ml of water was mixed with 20 ml of BPW (1:1 ratio) and previously collected floor swab which is retained in to BPW was also incubated overnight at the same temperature.

For the sake of selective enrichment 100 μl and 1000 μl of pre-enriched cultures were transferred into test tubes containing 9ml of Rappaport-Vassiliadis Enrichment Broth (OXOID England) and Tetrathionate broth (OXOID England), respectively and incubated overnight at 42 ^{o}C and 37 ^{o}C respectively. Samples showing turbidity from secondary both enrichment broths were then distinguished for the isolation purpose on selective and differential media.

Plating onto selective and differential media

A loop-full of suspension from both Rappaport-Vassiliadis Enrichment broth and Tetrathionate broth were streaked into plates of Xylose, Lysine, and Deoxycholate (XLD) (OXOID England). The plates were incubated at 37°C for 18-24hours and checked for growth of typical colonies of *Salmonella* which is red translucent and /or dome shaped colonies with black centers due to the production of hydrogen sulphide production by using Standard reference strains of S. Typhimurium (ATCC-13311) as quality control (**Appendix IX**). About 1- 3 suspected colonies per XLD plate were taken and streaked into slanted nutrient agar (OXOID England) to be incubated for 24 hours at 37^{o}C for further confirmation with biochemical analysis.

Confirmation with biochemical tests

Total of 95 stored samples in nutrient agar were transferred to tripticsoya broth (6 ml) to revive the bacteria before being streaked on to XLD to identify pure colonies. 1-2 pure colonies were then used to perform the intended biochemical tests including TSI, Urease, Lysine, Simmon's citrate, SIM, after preparation of mediums based on the manufacture instruction (**Appendix V).**

The ability of suspected colonies to ferment glucose, lactose, sucrose and H_2S production of isolated colonies was selected on prepared agar slant using a sterile straight wire loop. The center of the colony was lightly touched and the prepared TSI medium (OXOID) were inoculated by stabbing the butt and streaking the slants and then incubated at 37^oC for 24 hours. Colonies to split urea, through the production of the enzyme urease was inspected through Urea Agar (Blulux)**.** Surface of a urea agar slant was streaked with a portion of a well-isolated colony to be incubated at 37 °C for 24 hours. Lysine iron agar (OXOID) slants was used to test organisms for the competency to delaminate lysine or decarboxylate lysine. LIA was inoculated with a straight inoculating needle, stabbing through the center of the medium to the bottom of the tube and then streaking the slant. The tube was caped tightly and then incubates at 35°-37°C in ambient air for 18 to 24 hours. Simmons Citrate Agar (HIMIDIA) was used for the differentiation of suspected colonies based on the utilization of citrate as the sole source of carbon. Slopes were then stabbed and incubated at 37^o C aerobically for 24 hours. The final tests were Sulfur reduction, Indole production and motility tests through SIM medium (OXOID). The medium were stab down the center to within the bottom vertically by using inoculating needle

Finally, Colonies producing red slant (alkaline), with yellow butt (acid) on TSI with blackening due to hydrogen sulphide (H_2S) production and (gas production) in butt, negative for urea hydrolysis (yellow), positive lysine (Purple slant/purple butt (alkaline), with H_2Smasked butt reaction), positive for citrate utilization (deep blue slant) and, positive for sulfur reduction (blacking of medium) test and Lastly, positive sulfur reduction (blackening of the medium due to ferric sulfide),positive motility test

(obscure butt of the tube) and negative Indole test (yellow-brown ring) from SIM medium were considered to be *Salmonella* positive (**Appendix X**).

3.5 Data Management and Statistical Analysis

3.5.1. Development of biosecurity scoring system

Linear biosecurity scoring system was developed from the indicators of biosecurity events which were assessed through questionnaire. Which aids in integrating the level of biosecurity in each investigated farm with their exposure to infectious pathogens, with a particular focus on *Salmonella* prevalence (Kouam and Moussala 2018)

Questions related to both external and internal biosecurity measures (n=54) were sorted out from the entire questionnaire. Limited research comparing the effectiveness of biosecurity measures in mitigating disease transmission making it difficult to give weights to individual biosecurity measures. Consequently, biosecurity measures were considered equally important there by coded as 1 if biosecurity practice is present (implemented) or 0 if the practice is absent (not implemented) in unidirectional manner. Biosecurity measures which were expected to have a similar influence on the potential risk of introduction of contagious disease on the farm were combined into a single variable (**Appendix II).** Then, for each biosecurity variable group (made up of several measures) the values for each individual variable were added up to generate a biosecurity component (Greening and Rawdon, 2020 ; Soliman, 2020 ; Maduka *et al*., 2016;).

Finally ten basic biosecurity components were achieved then scored 0 to 10 since number of variable (question) in a given components were not equal (Steenwinkel *et al.*, 2011). After scaling of each component uniformly, the farms were considered as "Good/implemented" if the score of a given component is with a mean ≥ 5 per a given farm and "Not good/not implemented" if the component scores of the given farms is below mean < 5.).

3.5.2. Statistical data analysis

The raw data from the questionnaire survey was obtained as Excel files from the Kobo Toolbox server, and the laboratory results were entered into Microsoft Office Excel 2010 to be coded, processed, and imported into data analysis tools. Map of the study area was generated with ArcGIS v10.8.1 (ESRI, CA, USA).Descriptive statistics (frequencies, percentage, and mean standard deviation),level of adoption for good biosecurity score for each of ten components by multiple response analysis (MRA)and associations (through cross tab, chi-square (x^2)) were analyzed by using IBM SPSS v20 (IBM Corp., NY, USA). The farm level prevalence was calculated by dividing the number of positive farms by the total number of examined farms and samples level *Salmonella* prevalence was calculated by dividing total positive samples to total number of examined farms. P-value less than 0.05were considered significant with a 95% confidence interval.

3.6. Ethical Clearance

The study protocol was approved by research ethical review committee of Addis Ababa University College of Veterinary Medicine and Agriculture. The purpose and benefit of the study was clearly explained to participating individuals for the assessment of biosecurity and both oral and written consent form was taken at first place. Confidentiality of the participants and the farm was maintained by using unique code and sample collection was also competed according to animal welfare and safety requirements (Ref. N0. VM/ERC/09/04/13/2021) (**Appendix XI**).

4. RESULTS

4.1. Routines on Chicken Purchasing, Feeding and Watering Practices

Chicken purchasing routines of farms is illustrated in **Table 4**, feeding and watering of farms. 29 (51.8%) of them purchase day old chickens, 78.6 %were well- known chicken's supplier purchasers, 69.6% of farms doesn't perform inspection prior to purchasing, source of chicken suppliers are mostly vary (42.9%) and about 41(73.2 %) of farms can't deliver their stocks individually.92.9% of farms purchase feed from well-known producing companies and 51(91.1%) of them use tap water source.

Table 1: Routines on chicken purchasing practice, feeding and watering (n=56)

Biosecurity measures	Responses	N	P (%)
Type of chicken purchased	Day old chicken	29	51.8
	Pullets chicken	27	48.2
Source of purchased chicken	Well- known supplier	44	78.6
	Local chicken supplier	8	14.3
	Middle man	4	7.1
Delivery of chicken from same source	Yes, always	6	10.7
	No, sometimes vary	26	46.4
	No, mostly vary	24	42.9
Inspection routine while purchasing	Vent examination	5	8.9
	Random size and/or Wight	12	21.5
	No known inspection	39	69.6
Separate delivery of purchased chicken	Yes*	15	26.8
Source of feed	Purchased from companies	52	92.9
	In-house manual feed mix	4	7.1
Sealed feed storage against water	Yes*	33	58.9
Sealed feed storage against vermin	Yes*	16	28.6
Source of drinking water	Tap water	51	91.1
	Well water	1	1.8
	River water	4	7.1

*Number and percentages responded **"Yes".**

4.2. Routines on Management of Wastes and Farm Entry Restrictions

As illustrated in **Table 2,** only10 (17.9%) of farms have separated waste disposal area, 39 (69.6%) of farms doesn't use glove when they handle wastes also only 48.2% wash their hands after handling. Nonetheless, 67.9% of farm wastes were taken by dirt collectors. Farm entry restriction of 50 (89.3%) were weak since they are not obligatory for notification of visitors prior to their arrival, 54 (96.4%) farms doesn't

provide farm specific cloth, 20 (35.7%) of farms were reluctant to obligate visitors and farm workers to wash and disinfect their hands and 7 (12.5%) of farms has farm workers who also work for other poultry houses.

Table 5: Routines on management of wastes and farm entry restriction practices

Biosecurity measures	**Responses**	**N**	**P (%)**
Separated waste disposal area	Yes*	10	17.9
Way of handling wastes	Composting in pit	9	16.1
	Stored in sealed bage	14	25.0
	Immediat removal	10	17.9
	No recognized system	23	41.1
Useof gloves during waste handling	Always	1	1.8
	Sometimes	16	28.6
	Never	39	69.6
Habit of hand washing after waste handling	Always	27	48.2
	Sometimes	28	50.0
	Never	1	1.8
Destination of farm wastes	Disposed around farm	7	12.5
	Sell for others for other uses	11	19.6
	Taken by dirt collectors	38	67.9
Obligation of visitors to register	Yes*	1	10.7
Presence of farm specific cloths	Yes*	2	3.6
Hand washing and disinfecting before entering in to farm (visitors and farm workers)	Alwayes	6	10.7
	Sometimes	30	53.6
	Never	20	35.7
Precence of farm workers also working in another farm	Yes*	7	12.5

*Number and percentages responded **"Yes".**

4.3. Routines on Material Sharing, Farm Infrastructure and Biological Factors

As showed on as demonstrated by (**Table 3**) about 31 (55.4%) farms were found to have a reputation for sharing materials with other poultry farms, and only 16.1% of them have a routine of cleaning and disinfecting materials when they are received for use. Most farms have protective wall materials (44.6% brick wall and 51.8% mesh wire), which helps to avoid access of chickens to open air and access of pet animals 15 (26.8%).

Table 6: Routines on material sharing, farm infrastructure and biological factors (n=56)

Biosecurity measures	**Responses**	**N**	**P (%)**
Material being shared with other farms	Always, shared	6	10.7
	Sometimes, shared	31	55.4
	Never ,shared	19	33.9
Disinfect materials when receiving to use	Always	9	16.1
	Sometimes	26	46.4
	Never	21	37.5
Martial of chicken farm wall made of	Brick wall	25	44.6
	Mesh wire	29	51.8
	Soil plastered wall	2	3.6
Access of birds to outside (open air)	Yes*	15	26.8
Vegetation potentially harbor other animals	Yes*	51	91.1
Manifestation of vermin (e.g. rats, mice, etc.)	Yes*	55	98.2
Access of pet animals (cats and dogs)	Yes*	15	26.8

*Number and percentages responded **"Yes".**

4.4. Farm Relative Location and Disease Management

From the total farms, 55.4 % were within 100 meters of a four-wheeled vehicle access route, close to human residence area within 100m (82.1%) and close to neighboring poultry farms < 500m (66.1%). Concerning their disease management activities, 40 (71.4%) of farms have followed fixed vaccination program and 71.4% monitored flock health status every week or less even though 73.2% they did not achieve professional heath monitory service. On the other hand, only 5 (8.9%) of farms have isolation rooms and 37(66.1%) remove dead birds immediately after observation of death (**Table7**).

Table 7: Responses on farms relative location and disease management

Biosecurity measures	**Responses**	**N**	**P (%)**
Farm relative location from main road	main road with in 100m	31	55.3
	main road 100 -200m	23	41.1
	main road< 200 m	2	3.6
Residence area close to farm location	No house with in 100m	5	8.9
	House with in 100m	46	82.1
	house between 100 –200m	5	8.9
Approximate distance from nearest poultry farm	< 500m	37	66.1
	Between 500m and 1km	7	12.5
	> 1 km	12	21.4
Fixed vaccination program	Always followed	40	71.4
	Sometimes followed	16	28.6
Monitory of health status	Every week or less	40	71.4
	Every two weeks	11	19.6
	More than two weeks	5	8.9
Professional help for health status monitory	Privet animal health workers	4	7.2
	Government animal health worker	11	19.6
	By farm workers	41	73.2
Isolation of sick birds	Separated in isolation room	5	8.9
	Separated at corner of room with apparently healthy stocks	21	37.5
	No separation practice	30	53.6
Removal of dead bird	Immediately after observation	37	66.1
	Can be kept up to 24 hours	16	28.6
	Can be kept for > 24 hours	3	5.4

4.5. Routines on Cleaning and Disinfection Practices

As illustrated on **Table 8** majority of the farms 51(91.1%) have a habit of cleaning of the farm after each production cycle and about 23 (41.1) have foot bath but 20 (35.7%) access the farm without using it. About 33(58.9%) farms change footbath chemical every three or more days while only 22 (39.3) used to clean and disinfects farm materials.

Table 8: Responses on cleaning and disinfection practices

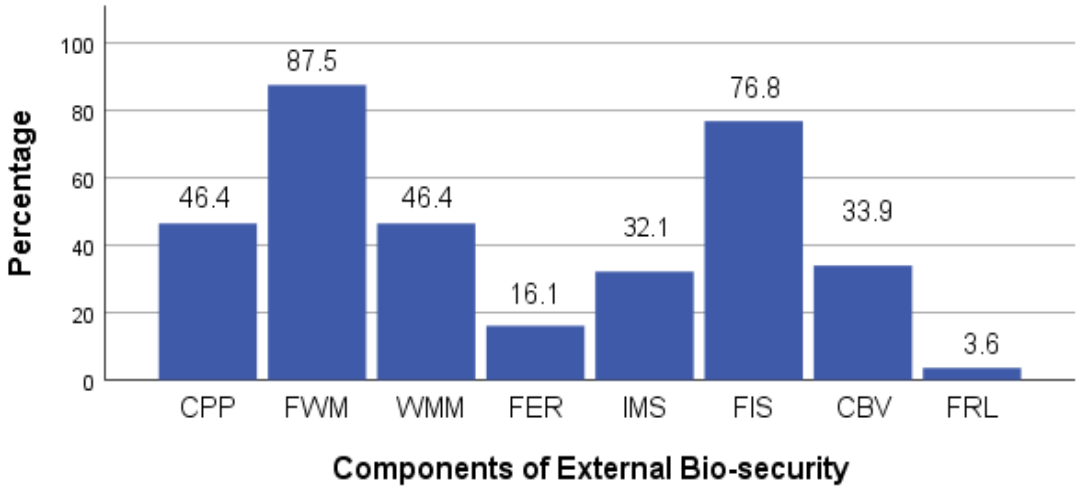
Biosecurity measures	**Responses**	**N**	**P(%)**
Cleaning of poultry farm after each production cycle	Always	51	91.1
	Sometimes	5	8.9
Presence of foot-bath facility	Yes*	23	41.1
	No	33	58.9
Probability of accessing farm without foot-bath	Never	36	64.3
	Sometimes	14	25.0
	Frequently	6	10.7
Frequency of changing foot-bath	Every three or more days	33	58.9
	Every two days	15	26.9
	Everyday	8	14.3
Cleaning and disinfection of farm materials	Yes *	22	39.3

*Number and percentages responded **"Yes".**

4.6. Adoption Level of Biosecurity Components

Result of multiple response analysis (MRA) is presented on **Figure 2**, depicting the extent of application of a certain external biosecurity component above the mean score (>5), to be scored as "Good" while **figure 3** depicts the internal biosecurity perspective. Feed and water management of the farms were highly implemented across the majority of farms 49 (87.5%), whereas relative location of the farm from

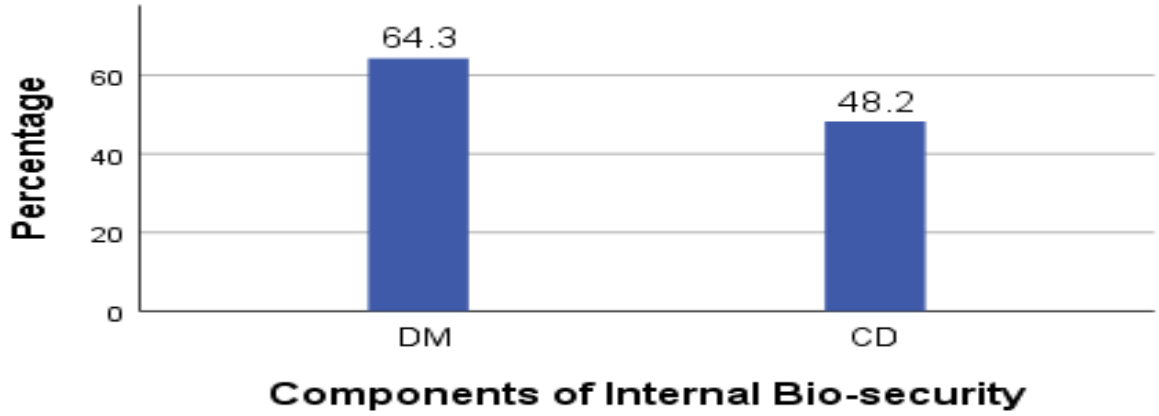
human residence areas, main road that can assist four-wheel vehicles, and nearby poultry farm was vastly violated by most of the farms, resulting in low implementation level of 2 (3.6 %).



CPP=Chicken purchasing practice, FWM= Feed and Water Management, WMM=Waste and Manure Management, FER= Farm Entry Restriction, IMS=Inter-farm Material Sharing, FIS=Farm Infrastructure, CBV= Control of Biological Vector, FRL=Farm Relative Location

Figure 3: Adoption level of external biosecurity components across total farms (n= 56)

From an internal biosecurity point of view, management of disease was implemented by 36 (64.3%) of farms, while cleaning and disinfection practices were implemented by 27 (48.2%) of total explored farms **(figure 4).**



DM=Disease Management, CD=Cleaning and Disinfection

Figure 4: Adoption level of internal biosecurity components across total farms (n= 56)

4.7. Sample Level Prevalence of Salmonella and Associated Risk Factors

Bacteriological investigation indicates that, out of total 391collected samples including pooled fecal drooping (223), floor swab (56), pooled feed (56) and drinking water (56), 24.3% samples were pronounced common characters of *Salmonella* species on XLD agar. However, only 5.6% of presumptive *Salmonella* isolates were confirmed through biochemical tests. As shown in **Table 9,** higher numbers of *Salmonella* isolates were found in fecal drooping (7.6%), small scale farms (5.1%), age of 7-12 chicken (3.1%), and breeds of Bovans brown (5.6%). Deep litter housing systems were relatively contaminated with presumptive *Salmonella* isolates (4.3%) when it is compared to poultry farms practicing cage system (0.5%) and mixed systems (0.8%), while high number of positive samples were also confirmed from layer poultry farms. From all farm characters there was a significant association in flock size and housing system with occurrence of *Salmonella* ($P< 0.05$).

Table 9: Sample level prevalence of *Salmonella* in poultry farms and associated risk factors in Addis Ababa (n=391).

Characteristics	Category	No. of samples examined	No. (%) of positive samples	X^2	P-value
Sample type	Fecal dropping	223	17 (7.6)	4.5	0.188
	Feed	56	1 (1.8)		
	Water	56	1 (1.8)		
	Floor swab	56	3 (5.4)		
Stock size	Small	252	20 (5.1)	7.1	0.008
	Medium	139	2 (0.5)		
Age of chicken in month	2-6	104	3 (0.8)	2.1	0.359
	7-12	187	12 (3.1)		
	>12	100	7(1.8)		
Breeds of chicken	Bovans brown	358	22 (5.6)	2.1	0.341
	Sasso	32	0 (0.0)		
	Mixed	1	0 (0.0)		
Housing system	Cage system	34	2 (0.5)	7.8	0.021
	Deep litter	344	17 (4.3)		
	Mixed	13	3 (0.8)		
Production Purpose	layer	347	21 (5.4)	1.1	0.305
	Broiler	44	1 (0.3)		

4.8. Farm-Level Prevalence and Associated Risk Factors

From the total 56 farms, 15 (26.8%) of farms were positive for *Salmonella* and with associated to farm characteristics, there were high *Salmonella* prevalence in layer farms (25.0%), small scale poultry farms (21.4%), farms which consisted 7-12 age

group (10.7%), Bovans brown breeds (26.8%) and deep litter systems(21.4%) (**Table 10**) There was no significant difference in *Salmonella* occurrence in all characters of farms except the housing system ($P < 0.05$).

Table 10: Farm level prevalence and associated risk factors

Characteristics	Category	No. of farms examined	No. (%) of positive samples	X^2	P-value
Production purpose	Layer	50	14 (25.0%)	0.4	0.554
	Broiler	6	1 (1.8%)		
Stock size	Small	42	12 (21.4%)	0.3	0.601
	Medium	14	3 (5.4%)		
Age of chicken	2-6	15	3 (5.4%)	1.3	0.500
	7-12	25	6 (10.7%)		
	>12	16	6 (10.7%)		
Breeds of chicken	Bovans brown	50	15 (26.8%)	2.5	0.293
	Sasso	5	0 (0.0%)		
	Mixed	1	0 (0.0%)		
Housing system	Cage system	7	1 (1.8%)	6.1	0.048
	Deep litter	48	13 (22.4%)		
	Mixed	1	1 (2.6%)		

4.9. Association of Farm Biosecurity Components and Salmonella Occurrence

Association of biosecurity components and *Salmonella* occurrence are illustrated in **Table 13**. Half of external biosecurity and both internal biosecurity components has significant association with the occurrence of *Salmonella* ($p<0.05$) and most of the farms with low score components of biosecurity were found to have high number of *Salmonella* isolates compared to farms that implement biosecurity measure at higher score.

Table 11: Association of biosecurity components and occurrence of *Salmonella*

Components of biosecurity	Score	No of farms examined	No. (%) of positive samples	X^2	P-value
External biosecurity					
Chicken purchasing practice	Bad	30	9 (16.1%)	0.34	0.391
	Good	26	6 (10.7%)		
water and feed management	Bad	7	5 (8.9%)	8.13	0.004
	Good	49	10 (17.9%)		
Waste & manure management	Bad	47	15 (26.8%)	3.92	0.048
	Good	9	0 (0.0%)		
Inter-farm material sharing	Bad	38	12 (21.4%)	1.38	0.239
	Good	18	3 (5.4%)		
Complete farm infrastructure	Bad	13	6 (10.7%)	3.23	0.072
	Good	43	9 (16.1%)		
Control of biological factors	Bad	37	13 (23.2%)	3.87	0.049
	Good	19	2 (3.6%)		
Relative location kept	Bad	54	15 (26.8%)	0.75	0.384
	Good	2	0(0.0%)		
Internal biosecurity					
Cleaning and disinfection	Bad	29	13 (23.2%)	10.9	0.002
	Good	27	2 (3.6%)		
Disease management	Bad	20	10 (17.9%)	9.9	0.001
	Good	36	5(8.9%)		

4. DISCUSSION

In the present study, 26 (46.4%) of farms implemented chicken purchasing practices above the mean score (>5) earning them a "Good" score. About 44 (78.6 %) of farms purchased their chicken from well-known chicken suppliers, but 24 (42.9%) of farms changed their purchasing source frequently because the suppliers were inefficient in providing enough chicken at requested time. Furthermore, 41 farms (73.2%) did not deliver the ordered flock individually. This result goes against Tanquilut *et al*., (2018), who assessed 80.7% of participating farms don't change chicken suppliers and most of the farms (83.3%) of farms receive their chicken individually. Such kind of variations might be arise from difference in farm managers knowledge and altitude about biosecurity practices related to chicken purchasing and delivery practices (Laroucau *et al.*, 2020).

A total of 49 (87.5%) of farms scored as "Good" in feed and water management. Similarly, Soliman, (2020) high biosecurity for feed and water sources, as well as quality care, were implemented between 2-3 (>75%) score groups, where 0 indicates complete absence of biosecurity and 3 indicates the high biosecurity implementation. This result may not otherwise be discovered through a pairwise analysis to Abdelgadir and Ismail (2014) whose have reported low implementation of effective biosecurity assurance on sources of feeding and guarding feed stores 16 (35.6 %) and water sanitation coupled with water system cleaning management 9 (20%).

Farm entry restrictions on assessed farms were extremely lax, only 9 (16.1%) of farms received a "Good" rating. The same is true of Greening and Rawdon, (2020) whose also indicated the movement of employees between sheds, with > 75% of enterprises having > 1 employee allocated to working through the whole farm. With a good adoption level of 2 (3.6 %) across the total farms, the relative placement of the farms from neighboring farms, human houses, and the major road were the first severely violated components of external biosecurity. The result is comparable with that of (Eltholth *et al.,* 2016). According to the authors, the compactness of poultry farms was very high. Chicken farms were found to be surrounded by other poultry farms within

a 500-meterradius. About 70% and 19% of farms were surrounded by broiler chicken farms and nearby laying chicken farms, respectively. However, the result of this study was very different with study conducted in developed countries such as Scotland on small and medium scale poultry farms. According to Correia-Gomes *et al*., (2021) assessment, most of respondents (>50% overall) had seldom or never seen neighbors' poultry, livestock and rodents, within 100 m.

Guide to chicken health and management in Ethiopia suggests that used litter, which comprises the original litter material, poultry manure, feathers and spilled feed, needs to be adequately disposed of, to minimize spread of disease (Habte *et al*, 2017). In this study, waste and manure management were implemented as good through 26 (46.4%) farms, which was similar with implementation level in chicken purchasing practices. Absence in separated wastes disposal area (82.1%), unable to handle in organized way (41.1%) and handling of wastes without gloves (69.6%) have impact on the indicated level of implementation score. The result is can agree with study conducted in Gharbia Governorate, Egypt by Eltholth *et al*. (2016) whose similarly reported About 75% of farms had no special designated area for poultry disposals and more than 85% never wear protective gloves or protective masks.

The overall infrastructure of most farms 43(76.8%) were adequate but 19 (33.9%) has a limitation to control of biological factors around the farm. Abah *et al.*, (2017) also mentioned bushy surroundings around poultry farms would allow breeding of insects and rodents. In present study, 51 (91.1%) declared the presence of vegetation with potential of harboring pet animals and vermin such as rats and mice. However, this result does not agree with the study conducted in United Arab Emirates on small scale poultry farms. The farm removes vermin (rats, mice,) and implements a vermin control program(97.3%),bird and vermin are denied access to the shades by fitting birds and vermin and maintain proof grids that are secured on the air inlets (100%) (Fathelrahman *et al*., 2020).

From internal biosecurity point of view, 36 (64.4%) have good implementation in management of disease. From those, 71.4 % has followed fixed vaccination program and frequent health status monitory but they were week in proper isolation strategies

since only 5(8.9%) of farms have separated isolation room for sick birds. These results concur with the findings of Asfaw and Ameni, (2021) whose have documented that encouraging status in disease diagnosis and vaccination programs (92.82%) in different parts of the country including Tigray, Amhara, Oromia and Addis Ababa regions. However the result is different from the report of Ezra *et al.,* (2020) in Kenya since most farmers (38.5%) do not vaccinate their birds against the common preventable diseases.

Cleaning and disinfection were rated as "Good" in 27 (48.2%) of the farms in this study. About 91.1% of them clean their production house after each production cycle. However, only 39.3 %clean and disinfect farm materials. Foot bath facility is available for 41.1% of farms but 35% of them have reputation to access the farm without using it. This finding agrees with study conducted in Egypt by Mohammed *et al*., (2016) whose investigated the poor utilization of permanent facilities for footbaths rather than using temporary plastic foot baths. However, this finding is different from a study conducted in the Debre Markos region of Ethiopia by Yitbarek and Mengistu, (2016) who reported that 77.6% of the small scale farms use footbaths.

Salmonellosis is one of the frequently reported diseases in Ethiopia, along with *New castle, Avian asterolosis, Coccidiosis, Salmonellosis, Pulorum disease,* and *Fowl pox,* which is considered as one of the major causes of reduce meat and egg production in poultry production (Goitom *et al*., 2017 ; Ziaul Haque *et al.*, 2021). This study showed 17 (26.8%) of farm level Salmonella prevalence out of 56 examined poultry farms. This result agrees with Dagnew *et al*. (2020) whose also reported 28.8% farm-level occurrence of *Salmonella* in central Ethiopia. However, this result is far lower than in the report from Nigeria79 (47.9%) by Jibril *et al*., (2020).

There was a significant difference in the occurrence of *Salmonella* between housing system and different age categories ($p<0.05$). The prevalence of *Salmonella* was increased with the increasing age of chickens. Generally, *Salmonella* in adult chickens might be attributed to the physiological stress of layers during egg-laying and molting, which depress their immune response and increase the susceptibility as compared to young chickens (Sarba *et al*., 2020). From a total of 391 samples, 5.6 %

of them were positive for *Salmonella* which is comparable to reports by Eguale, (2018) who also found 26 (4.7%) samples level prevalence from central Ethiopia. Even though, the result is lower than the study conducted in Modjo, central Ethiopia 31 (15.12%) by Abunna et al. (2016) and a study in southern Ethiopia, where *Salmonella* was isolated from 45 (16.7%) samples (Abdi *et al*., 2017). The observed differences in *Salmonella* prevalence among the studies could be attributable to differences in the sampled population, sample size, storage, processing, and isolation procedures used.

Salmonella occurrence varied significantly ($p < 0.05$) between farms with different stock sizes. Small flock farms experienced more isolates, this might be due to their weak biosecurity practice unlikely in developed member countries those have an accredited to implement specific control programs, which are lacking in developing countries like Ethiopia (Jibril *et al.*, 2020 ; Ziaul Haque *et al.,* 2021).There were also significant differences between housing systems ($p < 0.05$),with deeper litter resulted the high number of isolates (21.4%). Differences in the management system, such as the poultry housing system, stock size, and biosecurity efforts, as well as seasonal trends and/or pathogen survivability in the environment, could be the cause of such variation (Pires *et al.* 2019 ; Dailey *et al.* 2017).

Salmonella were frequently isolated from farms with a low biosecurity score than those farms with a high biosecurity score. Mridha *et al*. (2020) who evaluated the effectiveness of biosecurity-based training in preventing *Salmonella* bacterium infection backed up this viewpoint. Based on their results, the number of *Salmonella* isolates from project-intervened farms was lower (24.43%) compared to the number of *Salmonella* isolates from non-intervened farms (38.07%).

Feed and feed ingredients an act as a vector for the transfer of biological hazards. Consequently, a series of steps should be taken to help maximize feed biosecurity through assessing biological hazard risk, define protocols to prevent entry of hazard into the mill and utilize mitigation strategies to prevent risk(Stewart *et al*., 2020). In current study, there was a significant association between farms feed and water management and the frequency of *Salmonella* occurrences ($p<0.05$). Vermin (e.g.

rats, mice, etc.) were also nightmares to contaminate feed storage areas for most explored farms, 55 (91.1%) even if they purchased quality-assured feed from reputable feed suppliers in the country. Linked to this, there was a significant association between adoption level to control of biological factors and the prevalence of *Salmonella* ($p< 0.05$). Rodents are vectors and reservoirs for a wide range of pathogenic pathogens, including *Salmonella* spices, which has been particular concern for poultry breeders in recent years. Rodents contaminate animal feed in storage facilities and feeders mostly by their faces and urine (Amk and Sakthivel, 2015).

Waste and manure management practice of the farms were also found to be significant with the prevalence of *Salmonella* ($p<0.05$). Furthermore, 21.4 % of *Salmonella* prevalence was discovered on farms that implement waste and manure techniques incorrectly, compared to 5.4% on farms that execute them correctly. Improper waste disposal is thought to increase the risk of *Salmonella* infection in poultry farms because it allows for the reintroduction of *Salmonella* through fomites. Improper waste disposal was associated with a three-fold increase in the likelihood that the farm was positive for *Salmonella* (Jibril *et al*., 2020).

The study conducted by Greening and Rawdon, (2020) found movement of employees as one of the most frequent contact risk pathways for the introduction of pathogens across all of the surveyed farms that they have investigated. Similarly, there was a significant association ($p > 0.05$) between implementation of farm entry restrictions on farms and occurrences of *Salmonella* bacteria in this study. Additionally, *Salmonella* were absented in farms that implemented this strategy above the mean score.

To reduce bacterial loads and diseases on chicken farms, biosecurity and hygiene standards must be optimized through the use of extensive cleaning, disinfection, and lengthy sanitary barks lasting more than 14 days (Wibisono *et al*., 2020). In this study, cleaning and disinfection component of biosecurity found to have a significant relation with that of *Salmonella* occurrence ($p>0.05$) less amount of *Salmonella* isolate were resulted from farms those implemented cleaning and disinfection practice (5.4%) compering to farms those have not implement this biosecurity component

(17.9%). Hinojosa *et al*., (2018) support the same issue after they have investigated the power of high-pressure water rinse and foam additives are benefit in reducing aerobic bacteria and *Salmonella*.

Small-scale poultry farms are believed to have very limited biosecurity practices, use little vaccination, often host multiple poultry species and have higher contact rates with wild birds or foraging areas frequented by wild birds, which increases their susceptibility to diseases (Ezra *et al*., 2020). In current study, farm's disease management strategies were found to have strong significance relationship ($p<0.05$) with that of *Salmonella* occurrence. There was high prevalence of *Salmonella* in farms that did not implement the biosecurity component (17.9 %), but the percent of *Salmonella* incidence was (8.9 %) in farms who adopts biosecurity in good manner.

5. CONCLUSION AND RECOMMENDATIONS

The current study has provided a convenient data base on the biosecurity adoption level of small and medium scale poultry farms in Addis Ababa Ethiopia. As shown from the results, most of biosecurity components were not well adopted in the assessed farms. Principally, the concept of farm entry restriction and relative location of the farm were highly violated. Only 16.1 % of farms were scored "Good" in implementation of farm entry restrictions while 96.4% of the farms existed very close to main roads, human residence areas and neighboring poultry farms. On the other side, this study revealed 15 (26.8%) of farm level and total of 22 (5.6%) sample level *Salmonella* occurrence in the study region. According to the current finding, the possibility of recovering *Salmonella* Spp was higher on farms with a "Bad" implementation score for a given biosecurity component compared to those farms that implemented with a "Good" score..

Based on the above conclusion, the following recommendations were forwarded:

- Government should set biosecurity guidelines and strategies before establishing organizations such as poultry farm micro-enterprises in this sector.
- Higher institutional and Governmental Professionals should take the concept of biosecurity as principal issue and should create consecutive trainings and interventions.
- Low sore biosecurity in present study intended to be scaled up to ensure elimination of salmonella and other poultry pathogens in the farm
- Further investigations are required to gather information about occurrences and distributions of *Salmonella* serotypes and also antimicrobial resistance pattern.
- There should be encouraging rewards for the sake of motivating poultry farm owners with good enactments in management practices.

6. REFERENCES

Abah, H.O., Abdu, P.A., and Assam, A. (2017): Assessment of biosecurity measures against Newcastle disease in commercial poultry farms in Benue state , Nigeria. Sokoto *J. Vet. Sci*. **15**: 32–37.

Abdelgadir, A. and Ismail, H.(2017): Evaluation of Biosecurity Measures on Broiler Farms in Khartoum State , Evaluation of Biosecurity Measures in Layer Farms in Khartoum State , Sudan. *J. Appl. Sci. Res*. **5:** 23–31.

Abdi, R.D., Mengstie, F., Beyi, A.F., Beyene, T., Waktole, H., Mammo, B., Ayana, D., and Abunna, F.(2017): Determination of the sources and antimicrobial resistance patterns of *Salmonella* isolated from the poultry industry in Southern Ethiopia **17**: 1–12.

Abebe, E., Gugsa, G., and Ahmed, M.(2020): Review on Major Food-Borne Zoonotic Bacterial Pathogens. *J. Trop. Med*. **3**: 1–19.

Abunna F, Bedasa M, Beyene T, Ayana D, and Duguma R.(2016):*Salmonella*: Isolation and antimicrobial susceptibility tests on isolates collected from poultry farms in and around Modjo, Central Oromia, and Ethiopia. *J. Anim. Poult. Sci*. **5**: 21–35.

Aila, F.O.(2012):Biosecurity Factors Informing Consumer Preferences for Indigenous Chicken.*Business and Management Review.* **1**: 60–71.

Amk, M.R., and Sakthivel, P. (2015): Role of rodents in poultry environs and their management. *J. Dairy, Vet. Anim. Res.***2**: 107–114.

Andres, V.M., and Davies, R.H. (2015):Biosecurity Measures to Control *Salmonella* and Other Infectious Agents in Pig Farms : *A Review. Compr. Rev. Food Sci. Food Saf*. **14**: 317–335.

Antunes, P., Mourão, J., Campos, J., and Peixe, L.(2016): Salmonellosis : the role of poultry meat. *Clin. Microbiol. Infect*. **22**: 110–121.

Asfaw, Y., Asmeni, G., Medhin, G., Alemayehu, G., and Wieland, B.(2017): Infectious and parasitic diseases of poultry in Ethiopia : a systematic review and meta-analysis. *J. Poult. Sci*. **98**: 6452–6462.

Asfaw, Y. and Ameni, G.(2021): Poultry disease occurrences and their impacts in Ethiopia. *Trop. Anim. Heal. Prod*. **53**: 1–10.

Caekebeke, N., Jonquiere, F.J., Ringenier, M., Tobias, T.J., Postma, M., van den Hoogen, A., Houben, M.A.M., Velkers, F.C., Sleeckx, N., Stegeman, J.A., and Dewulf, J.(2020): Comparing Farm Biosecurity and Antimicrobial Use in High-

Antimicrobial-Consuming Broiler and Pig farms in the Beldian-Duch Border Region. *Front. Vet. Sci.***7**: 1-11

Can, M.F. (2018): Farm Level Biosecurity : Challenges and Suggestions. *J. Dairy Vet. Scciences.***7**: 5–8.

Chiroma, M.A., Adamu, S., Gadzama, J.J., Nelson, K.A., Hassan, A., Balami, A.G., Ya, M., and Bulama, I. (2018): Determination of egg production and weight in layers experimentally infected with *Salmonella* gallinarum. *African J. Cell. Pathol.***10**: 10–15.

Conan, A., Goutard, F.L., Sorn, S., and Vong, S.(2012). Biosecurity measures for backyard poultry in developing countries : a systematic review.*BMC veterinary research.***8***:*1-10

Correia-Gomes, C., Henry, M.K., Reeves, A., and Sparks, N.(2021): Management and biosecurity practices by small to medium egg producers in Scotland. *Poult. Sci.***62**:499-508.

CuC, N.T.K., DiNh, N.C., QuyeN, N.T. Le, TuaN, and ha miNh(2020): Biosecurity Level Practices in Pig and Poultry Production in Vietnam. *Adv. Anim. Vet. Sci.***8**: 1068–1074.

Dagnew, B., Alemayehu, H., Medhin, G., and Eguale, T.(2020): Prevalence and antimicrobial susceptibility of *Salmonella* in poultry farms and in-contact humans in Adama and Modjo towns, Ethiopia. *Microbiologyopen* **9**, 1–9.

Dailey, N., Niemeier, D., Elkhoraibi, C., Sentíes-Cué, C.G. and Pitesky, M. (2017): Descriptive survey and *Salmonella* surveillance of pastured poultry layer farms in California. *Poult. Sci.***96**: 957–965.

Eguale, T. (2018): Non-typhoidal *Salmonella* serovars in poultry farms in central Ethiopia : prevalence and antimicrobial resistance. *BMC Vet. Res*. **14**: 1–8.

Eguale T., Engidawork E., Gebreyes W. A., Asrat D., Alemayehu H., Medhin G., Johnson R. P. and Gunn J. S. (2016): Fecal prevalence, serotype distribution and antimicrobial resistance of Salmonellae in dairy cattle in central Ethiopia. *BMC Microbiol*. **16**: 1–11.

Eltholth, M.M., Mohamed, R.A., Elgohary, F.A., and Elfadl,E.A.A.(2016): Assessment of Biosecurity Practices in Broiler Chicken Farms in Gharbia Governorate , *Egypt. Alexandria J. Vet. Sci.* **49**: 68–77.

Ezra, B., Aondo, O., N, O.J., Joshua, O., Onduso, R., and Simion K, O.(2020): Poultry Farming and Disease Management Practices in Small- Scale Farmers in Kisii County, Kenya. *Glob. J. Sci. Front. Res. D Agric. Vet.* **20**: 1–9.

FAO (2019): Poultry Sector Ethiopia. FAO Animal Production and Health Livestock Country Reviews. No. 11. Rome.

Fathelrahman, E.M., Awad, A.I. El, Mohamed, A.M.Y., Eltahir, Y.M., Hassanin, H.H., Mohamed, M.E., and Hoag, D.L.K. (2020): and Small Farms in the United Arab Emirates. *agriculture.* **10**: 1–19.

Geer, D., Cocciolo, G., Circella, E., Pugliese, N., Lupini, C., Mescolini, G., Catelli, E., Stuhlträger, M.B., Zoller, H., Thomas, E., Camarda, A., (2020): Evidence of vector borne transmission of Salmonella enterica enterica serovar Gallinarum and fowl typhoid disease mediated by the poultry red mite , Dermanyssus gallinae. *Parasit. Vectors*. **13**: 1–10.

Gelaude, P., Schlepers, M., Verlinden, M., Laanen, M., and Dewulf, J.(2014): Biocheck.UGent: A quantitative tool to measure biosecurity at broiler farms and the relationship with technical performances and antimicrobial use. *Poult Sci*. **93**: 2740-2751.

Goitom, G., Bezabih E., and BerhanuG.(2017): Mapping Poultry Value Chain Functions in Adwa Wereda, Central Zone of Tigray, Ethiopia. *Ind. Eng. Lett*. **7**: 29–36.

Gosling, R.(2018): FARM PRACTICE: A review of cleaning and disinfection studies in. *Livestock*.**23**: 232–237.

Greening, S., Greening, S.S., Mulqueen, K., Rawdon, T.G., French, N.P., and Gates, M.C.(2020): Estimating the level of disease risk and biosecurity on commercial poultry farms in New Zealand Estimating the level of disease risk and biosecurity on commercial poultry farms in New Zealand. *N. Z. Vet. J*.**68**: 261–271.

Haftom, B., Alemayhu, T., Hagos, Y., and Teklu, A.(2015): Assessment of Biosecurity Condition in Small Scale Poultry Production System in and Around Mekelle , Ethiopia. *Eur. J. Biol. Sci*. **7**: 99–102.

Haggag, Y.N., Nossair, M.A., and Soliman, F.S.(2018): Assessment of Biosecurity Measures Applied in Infected Broiler Farms with Avian Influenza. *Alexandria J. Vet. Sci*.**56**: 107–113.

Habte, T., Amare, A., Bettridge, J., Collins, M., Christley, R. and Wigley, P. 2017. Guide to chicken health and management in Ethiopia. ILRI Manual 25. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Hergot, I.G., Rocha, C.M.B.M., Xavier, F.G., Santos, W.H.M., Oliveira, L.B. De, and Martins, N.R.S.(2021): Evaluation of actions of the official veterinary service to mitigate outbreaks of infectious laryngotracheitis and improve biosecurity on laying hen farms. *Brazilian J. Vet. Res*. **41**: 1–12.

Hinojosa, C., Caldwell, David, Byrd, J., Droleskey, R., Lee, J., Stayer, P., Resendiz, E., Garcia, J., Klein, S., Caldwell, Denise, Pineda, M., and Farnell, M.(2018): Use of foaming disinfectants and cleaners to reduce aerobic bacteria and *Salmonella* on poultry transport coops. *Animals***8:** 1–11.

https://biocheck.ugent.be/en

ISO (International Organization for Standardization) 6579 (2002): Microbiology of food and animal feeding stuff: horizontal method for the detection of Salmonella spp. Geneva

Jaleta, M.B., Abunna, F., Tufa, T.B., and Ayana, D.(2017):*Salmonella* : isolation and antimicrobial susceptibility tests on isolates collected from poultry farms in and around Modjo , Central Oromia , and Ethiopia.*J. Anim. Poult. Sci*. **5**: 21–35.

Jibril, A.H., Okeke, I.N., Dalsgaard, A., Kudirkiene, E., Akinlabi, O.C., Bello, M.B., and Elmerdahl, J. (2020): Prevalence and risk factors of *Salmonella* in commercial poultry farms in Nigeria. *PLoS One* **15:** 1–17.

Kebede, A., Kemal, J., Alemayehu, H., Habte and Mariam, S.(2016): Isolation, Identification, and Antibiotic Susceptibility Testing of Salmonella from Slaughtered Bovines and Ovines in Addis Ababa Abattoir Enterprise, Ethiopia: A Cross-Sectional Study. *Int. J. Bacteriol*. 1–8. doi:10.1155/2016/3714785

Kouam, M K, and Moussala, A.J.O., 2018. Assessment of Factors Influencing the Implementation of Biosecurity Measures on Pig Frams in the Western Highlands of Cameroon (Central Africa). Vet. Med. Int. 2018, 1-9.https://doi.org/10.1155/2018/9173646.

Lamas, A., Miranda, J.M., Regal, P., Vázquez, B., Franco, C.M., and Cepeda, A.(2018): A comprehensive review of non-enterica subspecies of Salmonella enterica. *Microbiol. Res.***206**: 60–73.

Laroucau, K., Zanella, G., Id, E.O., and Garcı, G. (2020): Characterization of commercial poultry farms in Mexico : Towards a better understanding of biosecurity practices and antibiotic usage patterns. *PLoS One***15**: 1–21.

Limbergen, T. Van, Dewulf, J., Klinkenberg, M., Ducatelle, R., and Gelaude, P.(2018): Scoring biosecurity in European conventional broiler production. *J. Poult. Sci*. **97**: 74–83.

Maduka, C. V, Igbokwe, I.O., and Atsanda, N.N. (2016): Appraisal of Chicken Production with Associated Biosecurity Practices in Commercial Poultry Farms Located in Jos , Nigeria. *scintifica.* 1–9. doi:10.1155/2016/1914692

Mcwhorter, A.R., Chousalkar, K.K., (2019): From hatch to egg grading : monitoring of Salmonella shedding in free - range egg production systems. *Vet. Res*. **50**: 1–9.

Mohammed, A.N., El, H., and Helal, S.(2016): Current situation assessment of biosecurity measures of some poultry sectors and hatcheries in Egypt. *OURNAL Vet. Med. Res.***23**: 143–154.

Morwal, S., Sk, S. (2017): Bacterial zoonosis - a public health importance. *J. Dairy, Vet. Anim. Res.* **5**: 56–59.

Mridha, D., Uddin, M.N., Alam, B., Akhter, A.H.M.T., Islam, S.K.S., Islam, M.S., Khan, M.S.R., and Kabir, S.M.L.(2020): Identification and characterization of *Salmonella* spp. From samples of broiler farms in selected districts of Bangladesh. *Vet. World***13**: 275–283.

NMSA. (2002). National Metrological Service Agency (2002). Year Book Bulletin.

Oluwasusi, J.O., Akanni, Y.O., and Sodiq, A.R.(2018): Effectiveness and Benefits of Biosecurity Practices in Small Scale Broiler Farmers in Ekiti State , Nigeria.*J. Poult. Res*. **15**:6–12.

Pham, P., Vinck, P., Kreutzer, T., Milner, J., Dorey, A., and Musaraj, P. KoBoToolbox| Data Collection Tools for Challenging Environments [Internet]. Kobo Toolbox. 2019.

Pires, A.F.A., Patterson, L., Kukielka, E.A., Aminabadi, P., Navarro-Gonzalez, N., and Jay-Russell, M.T.(2019): Prevalence and risk factors associated with Campylobacter spp. And *Salmonella* enterica in livestock raised on diversified small-scale farms in California. *Epidemiol. Infect*. **147**, 1–9.

Pulido-landínez, M.(2019): Food safety - *Salmonella* update in broilers. *Anim. Feed Sci. Technol*. **250**: 53–58.

Robertson, I.D.(2020): Disease Control , Prevention and On-Farm Biosecurity : The Role of Veterinary Epidemiology. *Engineering***6**: 20–25.

Sarba, E.J., Kudama, K., Dandecha, M., Megersa, L., Borena, B.M., and Gebremdhin, E.Z.(2020): Prevalence, organ distribution and antimicrobial susceptibility profile of *Salmonella* isolated from chickens purchased from markets in selected districts of West Shoa, Ethiopia. *Ethiop. Vet. J.***24**: 73–89.

Soliman, E.S. and, Abdallah, M.S.(2020): Assessment of biosecurity measures in broiler ' s farms in the Suez Canal area – Egypt using a seasonal prevalence of Salmonellosis. *Vet. World* **13**: 622–632.

Steenwinkel, S. Van, Ribbens, S., Ducheyne, E., Goossens, E., and Dewulf, J.(2011): Assessing biosecurity practices , movements and densities of poultry sites across Belgium , resulting in different farm risk-groups for infectious disease introduction and spread. *Prev. Vet. Med*. **98**:259–270.

Stewart, S.C., Dritz, S.S., Woodworth, J.C., Paulk, C., and Jones, C.K.(2020): A review of strategies to impact swine feed biosecurity. Anim. Heal. Res. Rev. **21**: 61–68.

Tanquilut, N.C., Espaldon, M.V.O., Eslava, D.F., Ancog, R.C., Medina, C.D.R., Paraso, M.G. V, Domingo, R.D., and Dewulf, J. (2018): Quantitative assessment of biosecurity in broiler farms using Biocheck . UGent in Central Luzon , Philippines. *Poult. Sci*. **99**: 3047–3059.

Tasie, C. M., Wilcox, G. I. and Kalio, A.E. 2020. Adoption Of Biosecurity For Disease Prevention And Control By Poultry Farmers In Imo State, Nigeria. *J. Agric. Food Sci.* **18**: 85–97.

Thrusfield, 2007. Veterinary Epidemiology, 3rd. Ed. Oxford: Blackwell Science. https://doi.org/10.1016/B0-72-169777-1/50023-8.

WHO. WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007–2015; World Health Organization: Geneva, Switzerland, 2015.

WHO. Who Global Foodborne Infections Network Laboratory Protocol, Isolation of Salmonella spp From Food and Animal Feaces. 5th ed; 2010.

Wibisono, F.M., Wibisono, F.J., Effendi, M.H., Plumeriastuti, H., and Rafi, A.(2020): A Review of Salmonellosis on Poultry Farms : Public Health Importance. *Syst. Rev. Pharm*. **11**: 481–486.

Wong, J.T., de Bruyn, J., Bagnol, B., Grieve, H., Li, M., Pym, R. and Alders, R.G.(2017): Small-scale poultry and food security in resource-poor settings: A review. *Glob. Food Sec.***15**: 43–52.

Yitbarek, M.B., and Mengistu, A.(2016): Disease management and biosecurity measures of small-scale commercial poultry farms in and around Debre Markos , Amhara Region , *Ethiopia. J. Vet. Med. Anim. Heal*. **8**: 136–144.

Zewdu, M. and, Wondimagegn, D.(2017): Review on Salmonellosis in Poultry and Its Public Health Importance. *Food Sci. Qual. Manag*. **61**: 47–61.

Ziaul H., Akter A.K.M., Islam M.R., Alam S.K.S., Neogi J., Yamasaki S.B., Lutful S., and Kabir, S.M.(2021): *Salmonella* gallinarum in small-scale commercial layer flocks: Occurrence, molecular diversity and antibiogram. *Vet. Sci*. **8**: 1–16.

7. APPENDICES

Appendix I: Questionnaire

Addis Ababa University

College of Veterinary Medicine and Agriculture

Questionnaire format to assess poultry farm biosecurity practices central Ethiopia

Instruction for the interviewer/enumerator:

- ✓ Choose the target respondent based on the following criteria. The respondent is preferably a person closely working with the chicken involving routine activities such as feeding, vaccination, cleaning of house, watering, collection of eggs and other similar activities.
- ✓ Read and explain for the study participant(s) the objective of the study using Afan Oromo or Amharic, as appropriate
- ✓ Ask for consent of the study participant to be involved in the study and ask to sign the consent form or give verbal consent, as appropriate.
- ✓ After getting signature or verbal consent, give one copy to the participant and retain one copy for file
- ✓ If she/he agrees, kindly request the respondent to find a place where he/she feels comfortable to answer the questions without much distraction.
- ✓ If the respondent will not agree to participate in the study for any reason, thank him/her and go to the next person/household.
- ✓ **Throughout the interviews:**
  - ➢ get organized and do not put yourself and the respondent in hurry; give enough time for the respondent to answer
  - ➢ don't give your opinion about the answer of any question; only be attentive with neutral expressions
  - ➢ in case the respondent asks you about your opinion on the topic, tell her/him to wait until the end of the questions
  - ➢ please check instruction indicated for each question on the right side in square bracket for some questions and act accordingly

Introduction and consent

Dear Respondent,

Good morning/good afternoon!

We are researchers from Addis Ababa University working poultry farm biosecurity (including hygienic practices) and also health management. We would like to ask you questions regarding overall management of chicken you are keeping. The questions will focus on three aspects: about your socio-demographic (as respondent), questions regarding feeding, watering, house, hygiene and drug use.

We would very much appreciate your participation in this survey and the information going to be collected is highly crucial for the for future works in the area of poultry health management and proper use of drugs in the poultry farms. We assure you that your answer will remain confidential and any of the information collected about your personal identity and the identity of your farm we will not be shared with third party. Your participation in this survey is voluntary and you can choose not to answer any individual question. However, as indicated above your participation and answering all of the questions in this survey is very important for us. The interview will last about 25 minutes.

Respondent agreement:

I have understood above statements and

☐ Agree to participate.

☐ Not agree to participate.

[For interviewer]:

- ✓ If no agreement, pass to the next respondent
- ✓ If the person cannot read and write, let him/her to give verbal consent. In that case, put the letter 'V' in the space provided for signature.

Name of respondent:_____ Signature_____

Name of the data collector_____ Signature_____

Start time _____ End time _____Date_____

Questionnaire survey to assess poultry farm biosecurity practices central Ethiopia

1. General characteristics of farm and respondent

1.1. Date of Interview (Date/month/year)_____

1.2. Name of the farm (If known by name): _____

1.3. Name of the respondent on farm _____

1.4. Role of Respondent on the farm

a) Farm owner b) Employee c) Owner relative

1.5. Age of farm respondent [should be more than 18 years to be eligible]

a) 18-25 b) 26-35 c) 36-45 d) >=46

1.6. Gender of the respondent

a. Male ☐ b. Female ☐

1.7. Up to which grade have you attended school?

a) None, no formal education b) Primary school

c) Secondary school d) Above secondary

2. Description of study farm area

2.1. Sub-city of the farm _____

2.2. Latitude and Longitude record (latitude_____/longitude_____)

3. Description of farm profile

3.1. What is the type (purpose) of the chicken farm?

a) Broilers b) Layers c) Multiple purposes

3.2. What Breed type kept in your poultry farm?

_____

3.3. Do you keep mixed breed type in your poultry farm?

a) Yes b) No

3.3 What is the age of the majority of the chicken you are currently keeping (in months?)

a) <=1 b. 2-6 c) 7-12 d) more than 13

3.4. Housing system /NB: observe the housing system and record or ask the respondent/

a) Deep litter system b) Cage system c) other (specify) _____

3.5. How many chicken are you keeping often (stock size of the farm)?

a) 100- 1000 (small scale) b) 1000-5000 (medium scale)

3.6. Are all previous batch of chicken removed before getting new one?

a) Yes, all time old ones are completely removed,

b) Yes, sometime old ones can remain on the farm,

c) I don't follow removing all the old chicken (all mixed together)

3.7. What other animal(s) are you keeping or owning?

a) Pig b) Horse c) Cattle d) Sheep

d) Goats e) Dogs/ cats f) Dove

3. chicken purchasing practices

4.1. What type of chicken do you purchase?

a) day-old chicken b) pullets c) other age groups

4.2. Where do you purchase the chickens?

a) Well known chicken suppliers

b) Local chicken supplier (based on availability)

c) Middlemen

d) Other (specify) _____

4.3. Is same source is used to get your chickens for this farm?

a) Yes, always the same supplier's b) No, Sometimes a different supplier

4.4. What routine inspection procedure you follow when purchasing chicken?

a) Vent examination

b) Random size and/or weight examination

c) No known inspection carried out

d) Other (specify) _____

4.6. Are the purchased chickens first delivered at your farm, i.e. before other farms are supplied by the same transport vehicle?

a) Always, after chickens delivered for others

b) Sometimes, get first

c) Always get first or individually delivered

5. Feed and water

5.1. What is your source feed?

a) Purchased layer/broiler feed

d) In-house manual feed mix

c) Other (specify) _____

5.2. Do you have a separate feed storage room?

a) Yes b) No

5.3. Are the feed storage area (storage of complete feed or concentrate) completely sealed against water?

a) Yes b) No

5.3. Are the feed storage area (storage of complete feed or concentrate) completely sealed against entry of rats or mice?

a) Yes b) No

5.4. What type of feeder for chicken is used on the farm?

a) Trough feeder b) Open container bin
c) Basic feed dispenser other

5.5. What is the source of water for your farm?

a) Well water b) Tap water c) Collected rain water
d) Waste water e) River water f) Other (specify) _____

5.6. What type of feeder for chicken you use on the farm?

a) Trough feeder b) Open container bin
c) Basic feed dispenser other

6. Manure and other wastes/litter management

6.1. Is there a separate storage area of manure and other wastes?

a) Yes b) No

6.2. How do you handle litter / waste on this farm?

a) Composting in pit
b) Store in sealed bag to remove
c) Immediately remove from floor a

d) No recognized system

6.3. When is waste/litter is removed?

a) After old batch and arrival of new batch
b) Occasional removal
c) Unknown

6.4. Do you use gloves when handling wastes?

a) Always b) Sometimes c) Never

6.4. Do you wash your hands after handling wastes if you did not use gloves?

a) Always b) Sometimes c) Never

6.7. What is done to the poultry waste (litter and manure)?

a) Dispose around farm location
b) Sell to others for other uses
c) Disposed by the farm somewhere else
d) Taken by dirt collectors
c) Other (specify) _____

7. Farm Entry Restrictions

7.1. Are visitors obliged to notify you of their presence before entering the poultry houses (e.g. Visitor's register?)

a) Yes b) No

7.2. Do visitors and farmworkers have to wear farm-specific shoes/overshoes before they are allowed to enter the poultry houses?

a) Yes, always b) Yes, sometimes c) No, they don't wear specific cloth

7.3. Do visitors and farmworkers have to wash and disinfect their hands before they are allowed to enter the poultry houses?

a) Yes, always

b) Yes, sometimes

c) No, they don't wash their hands before entering

7.4. Are there any other type of bird at home (e.g. dove)?

a) Yes b) No

7.5. Are there any farmworkers who also work on other poultry farms?

a) Yes b) No

8. Material Sharing, farm infrastructure, biological factor

8.1. Is there any material being shared with other farms that enters the poultry houses and/or has contact with your poultry?

a) Always, shared b) Sometimes, shared c) Never shared

8.2. Do you disinfect materials when receiving to be used on the farm? (e.g. disinfection with alcohol)?

a) Yes b) No

8.3. What is the wall of the chicken house made of?

a) Brick wall b) Net wall

c) Mesh wire d) Bamboo wall e) other (specify) _____

8.4. How is the status of entry to the chicken house (room)?

a) Fenced with door

b) Not fence (chicken has free movements inside the compound

8.5. From which material the floor of the farm is made of?

a) Concrete floor b) Brick sealing

c) Soiled floor

8.6. Does the poultry have access to the outside, i.e. the open air?

a) Yes b) No

8.7. Is there a possibility that wild birds enter the poultry houses?

a) Yes b) No

8.8. Are there vegetation which can potentially harbor other animals such as mouse and cats around the farm?

a) Yes b) No

8.9. Are vermin (e.g., rats, mice, etc.) considered to be a problem on the farm?

a) Yes b) No

8.10. Do dogs or cats have access to the poultry house?

a) Yes b) No

10. Relative location of the farm

10.1. Is there a main road (more than 15 meters wide) which can be accessed by four-wheeled vehicle near to your farm gate?

a) Yes, main road with in 100 m

d) No main road near to the farm (less than 200m)

c) Yes, road between 100-200m

10.2. Is there human residence area near the location the farm (N.B: ask or observe)

a) No house within 100meter b) Houses within 100meter

c) House between 100-200meters

10.4. At what approximate distance (straight-line) is the nearest neighboring poultry farm located?

a) Less than 500 meters (Less than 0.3 miles)

b) Between 500 meters and 1 kilometer (between 0.3 and 0.6 miles)

c) More than 1 kilometer (more than 0.6 miles)

11. Poultry disease management

11.1. Is there a fixed schedule or program for vaccinations?

a) Yes b) No

11.2. How often the health status of the chicken on the farm is monitored?

a) Every week or less b) every two weeks c) More than two weeks

11.3. By whom is the health status of the chicken on the farm is monitored?

a) Private animal health workers

b) Government animal health worker

c) By farm workers (not trained)

11.3. How frequent are the dead birds removed from the poultry house?

a) Daily, immediately after observing death of the birds

b) Every two days

c) Less frequent than once two days

11.4. Are there different ages categories of poultry present on your farm?

a) Yes b) No

11.5. Commonly, how are sick birds handled?

a) Separated in separated room dedicated for this purpose
b) Separated in corner of room where other apparently normal birds are kept
c) No separation practiced

12. Cleaning, disinfection and sanitation measures

12.1. Are the poultry houses cleaned after each production cycle if you following separate cycle?

a) Always b) Sometimes c) Never

12.2. Are the poultry houses disinfected after each production cycle?

a) Always b) Sometimes c) Never

12.3. How long does the sanitary break after each production cycle last?

a) Less than 3 days b) between 3 and 8 days c) More than 8 days

12.6. Is there a disinfection footbath at the entrance of the farm?

a) Yes b) No

12.7. Which chemical is used for footbath?

a) Berekina b) Formalin c) Other (specify) _____

12.8. Is there a means to access the farm or get into the farm without stepping on footbath?

a) Yes, people can have access without foot bathing most often
b) Sometimes people can access without foot bathing
c) Never, people use always foot bathing to access

13.9. How often is the water and disinfectant of the foot bath changed?

a) Every day b) Every two days c) more than every three days

12.10. Do you practice cleaning and disinfection of drinkers both on the in- and outside after each production cycle?

a) Yes b) No

Appendix II: Biosecurity Variables combined in to Single component

Component 1: Chicken Purchasing Practices

- Keeping different chicken breeds on the given farm
- Application of all in all out system
- Purchasing chicken from well-known/assured supplier
- Permanent source of chicken supplier
- Inspection of chicken while purchasing
- Separated receive of chicken for a given farm

Component 2: Feed and Water Managements

- Purchase feed from assured source of feed
- Presence of separated feed storage area
- Use of a feeder that is free of contamination
- Use of clean water source

Component 3: waste and Manure Managements

- Availability of separated manure and waste storage area
- Proper handling of litter/waste on the farm
- Use of gloves when the time of waste/litter handling
- Routine of hand washing after handling of wastes
- Appropriate disposal system for wastes and manure

Component 4:Farm Entry Restrictions

- Notification/ registration of visitors prior to their arrival
- Presence of farm specific cloths for visitors and farm workers
- Hand washing and disinfecting before entering in to farm
- Precence of farm workers also workingin another farm

Component 5: Inter-farm material sharing

- Sharing of any material with other poultry farms
- Disinfection of shared materials while receiving

Component 6: Farm Infrastructures

- Material of farm wall (Brick and mesh walls)
- Restricting chicken house with fenced door
- Material of poultry house (concrete or Brick sealing)

Component7: Control of biological vectors

- Access of poultry to outside/open air
- Access of wild birds enter in to poultry house
- Access of pate animals (Dogs and Cats)
- Presence of vegetation which can harbor other animals
- Problem of vermin e.g. rats, mice, etc.

Component8: Farm Relative location

- Relative location of farm from main road
- Relative location of farm from human residence area
- Relative location of farm from nearest neighboring farm

Component 9: Disease Managements

- Fixed vaccination program/schedule
- Frequency of health status monitory
- Professional health status monitory
- Isolation of sick birds
- Removal of dead birds

Component 10: Cleaning, Disinfections. And Sanitation Measures

- Cleaning of Chicken house after each production cycle
- Sanitary brake after each production Cycle
- Presence of disinfection footbath at the entrance of the farm
- Use of disinfection footbath chemicals
- Means to assess the farm without stepping footbath
- Frequency of changing disinfectant of footbath
- Cleaning and disinfection of farm materials after each production cycle

Appendix III: Total score of assessed farms (n=56)

Farm_ID	CPP	FWM	WMM	FER	MS	RIS	CBV	FRL	DM	CD	Total (100 %)
B6	5.7	8.3	5	6	3.3	10	5	3.3	7.1	4.3	58
B7	7.1	8.3	5	2	3.3	10	5	0	5.7	5.7	52.1
B5	2.9	8.3	5	4	6.7	10	2.5	0	4.3	4.3	48
L2	2.9	5	3.3	2	3.3	3.3	2.5	0	8.6	8.6	39.5
L1	5.7	3.3	3.3	4	6.7	6.7	0	3.3	2.9	7.1	43
L3	4.3	8.3	3.3	4	3.3	0	2.5	3.3	7.1	4.3	40.4
G2	5.7	6.7	1.7	4	3.3	10	2.5	0	4.3	4.3	42.5
A2	8.6	5	5	4	6.7	3.3	2.5	3.3	7.1	5.7	51.2
A1	4.3	8.3	5	4	3.3	3.3	2.5	3.3	7.1	2.9	44
A5	4.3	5	1.7	4	6.7	6.7	0	3.3	5.7	4.3	41.7
A3	8.6	8.3	5	6	3.3	10	7.5	3.3	5.7	2.9	60.6
G3	4.3	3.3	3.3	2	3.3	3.3	0	3.3	7.1	2.9	32.8
G5	5.7	5	1.7	4	3.3	10	2.5	0	5.7	4.3	42.2

G4	5.7	6.7	3.3	2	3.3	6.7	2.5	3.3	10	4.3	47.8
A4	4.3	5	3.3	4	3.3	10	5	3.3	5.7	2.9	46.8
G1	4.3	6.7	3.3	2	3.3	10	2.5	0	5.7	4.3	42.1
K1	7.1	5	3.3	2	3.3	10	2.5	0	7.1	7.1	47.4
K3	4.3	6.7	5	6	3.3	10	2.5	0	8.6	5.7	52.1
K6	4.3	5	5	2	3.3	10	7.5	0	7.1	5.7	49.9
N2	5.7	6.7	1.7	4	3.3	10	2.5	0	2.9	1.4	38.2
N3	4.3	6.7	3.3	2	3.3	10	2.5	3.3	4.3	7.1	46.8
N4	5.7	5	3.3	2	3.3	3.3	5	3.3	4.3	4.3	39.5
N5	4.3	6.7	5	2	3.3	10	5	0	2.9	4.3	43.5
N7	5.7	6.7	3.3	4	3.3	3.3	2.5	3.3	7.1	7.1	46.3
N6	4.3	6.7	3.3	4	3.3	0	0	3.3	2.9	4.3	32.1
K8	5.7	6.7	5	4	6.7	10	2.5	3.3	7.1	8.6	59.6
N1	2.9	3.3	3.3	4	6.7	10	2.5	3.3	5.7	5.7	47.4
Y1	4.3	6.7	5	6	6.7	10	2.5	3.3	7.1	5.7	57.3
Y2	8.6	5	1.7	2	10	0	5	6.7	5.7	2.9	47.6
Y3	7.1	8.3	6.7	4	10	10	5	0	7.1	5.7	63.9
KI1	4.3	1.7	5	2	3.3	10	0	0	4.3	5.7	36.3
KI2	4.3	6.7	5	2	3.3	10	2.5	0	4.3	4.3	42.4
K2	5.7	6.7	3.3	2	3.3	10	2.5	3.3	4.3	7.1	48.2
K5	2.9	6.7	1.7	2	3.3	10	5	0	7.1	8.6	47.3
K7	5.7	1.7	1.7	4	3.3	3.3	2.5	3.3	4.3	2.9	32.7
K4	4.3	5	3.3	6	3.3	6.7	2.5	0	7.1	4.3	42.5
K9	1.4	5	0	2	6.7	3.3	2.5	0	4.3	1.4	26.6

AK1	2.9	8.3	6.7	2	6.7	6.7	2.5	0	4.3	4.3	44.4
AK3	4.3	8.3	3.3	6	6.7	10	0	0	4.3	4.3	47.2
AK2	4.3	6.7	5	4	3.3	10	5	0	7.1	4.3	49.7
Y7	5.7	6.7	5	4	3.3	3.3	5	3.3	7.1	8.6	52
Y4	4.3	6.7	1.7	4	3.3	6.7	2.5	0	5.7	4.3	39.2
Y5	4.3	3.3	5	2	6.7	6.7	5	3.3	4.3	5.7	46.3
Y6	7.1	0	3.3	4	3.3	0	2.5	3.3	5.7	4.3	33.5
B1	5.7	10	1.7	6	6.7	10	7.5	0	5.7	8.6	61.9
B2	4.3	8.3	6.7	2	3.3	10	2.5	0	4.3	7.1	48.5
B3	2.9	8.3	3.3	2	3.3	10	5	0	4.3	5.7	44.8
B4	1.4	8.3	5	4	3.3	10	5	0	2.9	7.1	47
KO1	5.7	8.3	5	4	10	10	2.5	0	8.6	4.3	58.4
KO2	4.3	8.3	5	4	3.3	10	2.5	0	5.7	4.3	47.4
KO3	5.7	8.3	6.7	4	3.3	6.7	0	0	7.1	4.3	46.1
KO4	7.1	10	6.7	10	10	10	5	0	7.1	8.6	74.5
KO5	1.4	10	5	8	10	10	2.5	0	8.6	7.1	62.6
KO6	5.7	8.3	5	4	3.3	10	5	3.3	7.1	5.7	57.4
KO7	5.7	8.3	1.7	4	10	10	5	3.3	4.3	5.7	58
N8	5.7	8.3	0	4	3.3	10	0	0	7.1	5.7	44.1

CPP= Chicken Purchasing Practices, FWM= Feed and Water Management, FER= Farm Entry Restriction, MS=Material Sharing, FIS=Farm Infrastructure, CBV=Control of biological Vectors FRL=Farm Relative Location, DM=Disease Management, CD=Cleaning and Disinfection

Appendix IV: Sample Collecting and Recording Sheet

Sample code	Farm Id	Site of collection	Farm location (GPS point)	Date of collection	Sample type	Result on non-selective enrichment media (BPW)	Result on selective enrichment media (RV,TT)	Culture result on XLD agar	Result for biochemical tests				
									TSI	**UREA**	**LYSIN**	**CITRATE**	**SIM**

Appendix V: Types of media and reagents used for isolation and biochemical test with their preparation.

Buffered Peptone Water Preparation

Preparation: 20 grams of BPW components were suspended in 1000ml of distilled water Mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test and sterilize in autoclave at 121 ℃ for 15 minutes. Final PH is 7.0± 0.2 at 25℃.

Composition (g/l):

Peptone……10.00
NaCl……5.00
Na2HPO4……3.50
KH2PO4……...1.50

Rappaport Vassiliadis *Salmonella* Enrichment Broth

Preparation: 49.17 grams of hydrated medium RVS broth were suspended in 1000ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as

desired in to tubes and sterilize by autoclaving at 115^{o}c for 15 minutes, final PH 5.2 ± 0.2 at 25°C.

Composition (g/l):

Soya peptone …….4.5
Sodium chloride ……7.20
Potassium dihydrogen phosphate …….1.44
Magnesium chloride ……..36
Malachite green …….0.036.

Tetrathionate Broth

Preparation: Suspend 77.40 grams in 980 ml purified/ distilled water. Heat just to boiling, Cool below 45-50°C and aseptically add 20 ml iodine solution (6 grams of iodine and 5 grams of potassium iodide in 20 ml distilled water). Mix well and dispense in 10 ml quantities in sterile tubes. This complete medium should be used on the day of preparation. Do not heat after the addition of iodine solution. Use the medium immediately after addition of iodine.

Composition (g/l):

Meat extracts B #..... 0.900
Peptone …… 4.500
Yeast extract……. 1.800
Sodium chloride…… 4.500
Calcium carbonate……. 25.000
Sodium thiosulphate…….. 40.700

Xylose Lysine Deoxycholate Agar (XLD)

Preparation: Suspend 56.68 grams in one liter of distilled water. Heat with frequent agitation until the medium boils. **DO NOT OVER HEAT**. Transfer immediately to a water bath at 50℃. Pour in to plates as soon as the medium has cooled. It is important to preparing large volumes which will cause prolonged heating. Final PH: 7.4 ± 0.2 at 25 ℃.

Composition (g/l):

Yeast extracts …….3.0
L-lysine hydrochloric acid …….5.0
Xylose …….3.5
Sodium desoxycholate…….2.5
Lactose ……7.5
Sucrose ……...7.5
L-Lysine hydrochloride ……..…5.0
Sodium chloride ……..…5.0
Sodium thiosulphate……6.8
Ferric ammonium citrate …….0.8
Phenol red …….0.08
Agar ……...15.0

Nutrient Agar

Preparation: suspend 28 grams powder in 100ml distilled water. Then Heat, to boil and to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121℃) for 15 minutes. Mix well and pour in to sterile Petri dishes. Final Ph, is7.4 ± 0.2 at 25°C.

Composition (g/l):

Agar……...….15.0,
Peptone……5.0,
Sodium chloride …….5.0,

Yeast extract……2.0
Beef extract……1.0.

Triple sugar agar

Preparation: suspend 64.2 grams dehydrated medium in 1000ml of distilled water. Bring to boil to dissolve completely. Mix well and distribute in to containers. Sterilize by autoclaving at 10lbs pressure or 115°C for 30 minutes. Allow the medium to set in sloped form with a butt about 2.5cm long. Final PH: 7.4 ± 0.2 at 25°C.

Composition (g/l):

Beef extract …….3.0
Yeast extract ……3.0
Peptone ……20.0
Sodium chloride…… .5.0
Lactose ……10.0
Sucrose ……10.0
Dextrose monohydrate ……1.0
Sodium chloride ……5.000
Ferric sulphate ……0.2
Sodium thiosulfate …….0.3
Phenol red …….0.024
Agar ……12.0

Simmon's Citrate Agar

Preparation: Suspend 24.28 grams in 1000 ml distilled water. Then Heat, to boiling, to dissolve the medium completely. Mix well and distribute in tubes or flasks. For tubes, dispense 4.0 to 5.0 ml into 16-mm tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool in slanted position (long slant, shallow butt). Tubes should be stored in a refrigerator to ensure a shelf life of 6 to 8 weeks. The

uninoculated medium will be a deep forest green due to the pH of the sample and the bromothymol blue. Final pH (at 25°C) 6.8±0.2

Composition (g/L):

Magnesium sulphate …….0.200
Ammonium dihydrogen phosphate …….1.000
Dipotassium phosphate ……1.000
Sodium citrate …….....2.000
Sodium chloride ……...5.000
Bromthymol blue …….0.080
Agar …….15.000

LYSINE IRON AGAR

Preparation: Suspend 34g in 1 liter of distilled water. Bring to the boil to dissolve completely. Dispense into tubes and sterilize by autoclaving at 121° C for 15 minutes. Cool the tubes in an inclined position to form slants with deep butts.PH 6.7 ± 0.2 at 25°C

Composition (g/l):

Bacteriological peptone……5.0
Yeast extract……3.0
Glucose……1.0
L-lysine……10.0
Ferric ammonium citrate……0.5
Sodium thiosulphate…….0.04
Bromocresol purple……...0.02
Agar……..14.5

SIM (Motility-Indole Medium)

Preparation: Suspend 30g in 1 liter of distilled water and boil to dissolve the medium completely. Dispense into final containers and sterilize by autoclaving for 15 minutes at 121°C. Final pH, 7.3 ± 0.2 at 25°C

Composition (g/l):

Tryptone……20.0
Peptone……6.1
Ferrous ammonium sulphate…….0.2
Sodium thiosulphate……..3.5

UREA Agar Base

Preparation: Suspend 2.4g in 95ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115°C for 20 minutes. Cool to 50°C and aseptically introduce 5ml of sterile 40% Urea Solution SR0020. Mix well, distribute 10ml amounts into sterile containers and allow setting in the slope position. Final pH 6.8 ± 0.2.

Composition (g/l):

Peptone……...1.0
Glucose…….……1.0
Sodium chloride…….....5.0
Disodium phosphate……..…1.2
Potassium dihydrogen phosphate……. 0.8
Phenol red…….0.012
Agar…….15.5

Tryptone soya broth

Preparation: suspend 30 g to 1 litter of distilled water, mix well and distribute into final containers. Then Sterilize by autoclaving at 121°C for 15 minutes. Final pH 7.3 ± 0.2

Composition (g/l):

Pancreatic digest of casein ……17.0
Papaic digest of soybean meal ….....3.0
Sodium chloride ……5.0
Dibasic potassium phosphate ……2.5
Glucose …….2.5

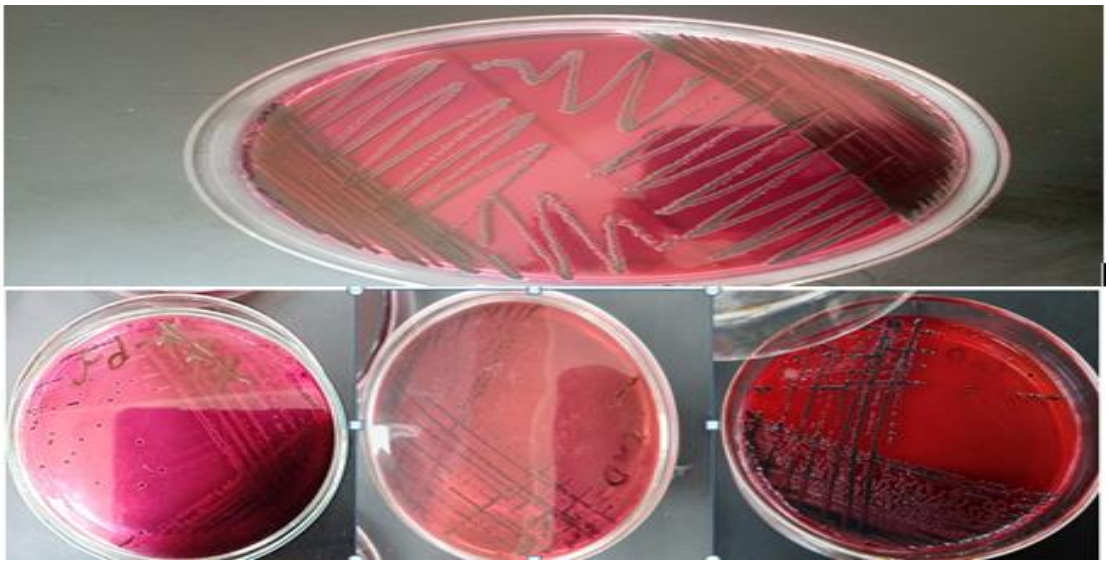
Appendix VI: External and Internal Futures Favoring Vermin and Pets

Appendix VII: Relative Location of Farms from other Farms and Human Residence

Appendix VIII: Pictures taken during sample collection and laboratorial activities

Picture during sample collection (B and C) and picture during Lab. Activities (A and D).

Appendix IX: Pictures Showing Colony Character of *Salmonella* on Selective (XLD) Media



Appendix X: Pictures Showing Biochemical Results

Salmonella positive and negative on TSI (1A, 1B), *Salmonella* positive and negative on Urea Agar Base (2A, 2B), *Salmonella* futures on SIM Media (3A) comparing to pure negative (3B), *Salmonella* positive and negative on Lysine Iron Agar (4B, 4A) and *Salmonella* positive and negative on Simmon's Citrate Agar (5B, 5A).

Appendix XI: **Ethical clearance of AAU-CVMA**

አዲስ አበባ ዩኒቨርሲቲ
የእንስሳት ሕክምናና
ግብርና ኮሌጅ
ቢሾፍቱ

ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: Certificate Ref. No: VM/ERC/09/04/13/2021

Name of Applicant: **Hika Waktole_ (BSc, MSc, Assit. Professor of Vet. Microbiology)**

Address: **Department of Microbiology, immunology and Vet. Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University**

Title of the project: *Biosecurity practices in Poultry Farms: isolation, identification and molecular characterization of major bacterial pathogens, investigation of major bacterial zoonosis and biosecurity based interventions towards enhancing production efficiency and profitability in poultry farms in central Ethiopia*

Date of application:	**March, 2021**
Nature of the project:	**Mildly invasive /little stress**
Target animal species:	**Domestic chicken**
Number of animals involved:	**5760**
Study area:	**Central Ethiopia, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/13/021, 21/04/2021**

The above mentioned 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
1. Any major study on human subjects (except questionnaire survey) should get a separate clearance from relevant bodies

Getachew Terefe (DVM, PhD) _____
Chairman Signature

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

መልሱን በሚጽፉልን ጊዜ እባክዎን የኛን ደብዳቤ ቁጥር ይጥቀሱልን
Please quote Our Ref. No. When Replying

ፋክስ} ስልክ } ፖ.ሣ.ቁ
Fax 251-11-4339933 Tel. +251 114338450 P.O.Box}34 ቢሾፍቱ፤ ኢትዮጵያ
Bishoftu, Ethiopia