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COLLEGE OF VETERINARY SCIENECES

COCCIDIOSIS IN BROILER FARMS OF MEKELLE CITY, NORTHERN ETHIOPIA: ANALYSIS OF BIOSECURITY PRACTICES, PREVALENCE, AND POSTMORTEM LESIONS

BY

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DECLARATION

I hereby declare that this thesis titled "**Coccidiosis in Broiler Farms of Mekelle City, Northern Ethiopia: Analysis of Biosecurity Practices, Prevalence, and Postmortem Lesions**" represents my independent work. It does not include, without acknowledgment, any material previously submitted for a degree or diploma at any university. To the best of my knowledge, it does not contain any material previously published or authored by another individual, except where proper references are provided in the text. All significant contributions made by others to this work, including jointly authored publications, have been clearly acknowledged.

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DEDICATION

I dedicate my graduate work to my family, especially my beloved wife and children, for their unwavering love, understanding, and support throughout this program. Their sacrifices have been countless, and their encouragement has been a source of strength during the most challenging moments. Each late night spent studying, each moment of self-doubt, was eased by the warmth of their belief in me. The laughter and joy of my children reminded me of what truly matters, and my wife's patience and unwavering support fortified my resolve. I am profoundly grateful for their presence by my side, the many sacrifices they made, and the way they cheered me on every step of the way. This achievement is as much theirs as it is mine, and I hope to repay their faith with a life filled with love, joy, and shared accomplishments. Thank you for inspiring me to reach for my dreams and for being my greatest champions in this endeavor.

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LIST OF ABREVIATIONS

DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agricultural Organization
GIT	Gastro Intestine Tract
IgG	Immunoglobin G
IgM	Immunoglobin M
OPG	Oocyst Per Gram
PCR	Polymerase Chain Reaction
USDA	United States Department of Agriculture

ABSTRACT

Coccidiosis, a serious parasitic disease, poses a significant threat to commercial chicken farms, leading to substantial financial and job losses for producers. This study aimed to quantify the relationship between poultry coccidiosis prevalence and biosecurity practices on commercial broiler farms. A cross-sectional study was conducted from January to November 2024 assessed biosecurity levels and coccidiosis prevalence in small and medium-sized broiler farms in Mekelle city. A total of 257 fresh chicken dropping samples from broiler farms were collected and examined for Eimeria oocysts using microscopy and McMaster Technique, and biosecurity practices of 23 small and 15 medium-scale farms were measured using UGBioCheck Tool. The overall prevalence of Eimeria oocyst infections among the fecal samples were 23.74% and farm-level prevalence was 68.42 %. The analysis revealed a significant association between age and coccidiosis prevalence ($p < 0.05$), indicating that younger chickens (< 8 weeks) had a higher infection rate compared to older ones. The study also categorized the oocyst counts, and 70.49% of positive samples had low levels of infection (less than 10,000 oocysts per gram), while only 9.84% exhibited high levels of infection. In postmortem examinations, 55.56% of the examined cases showed gross lesions indicative of coccidiosis, with the cecum being the most affected site (38.89%). The overall, external and internal biosecurity scores were measured to be 47.26%, 44.57% and 53%, respectively. This shows that the biosecurity scores were significantly lower than global averages. Furthermore, external biosecurity score was lower than internal biosecurity score, with visitors and farm workers and disease management rated highest in external and internal biosecurity. Over 73% of the farms housed other animals, and 42.11% reported nearby water sources that could risk disease transmission. All farms followed vaccination protocols and removed dead birds daily, with around 97.37% cleaning poultry houses after each production cycle. The study found a strong correlation between low biosecurity scores and higher occurrences of coccidiosis; specifically, 57.89% of farms with poor biosecurity scores tested positive for Eimeria oocysts. The findings underscore the need for improved biosecurity practices to enhance poultry health and reduce disease prevalence within broiler farms.

Keywords: *Biosecurity, Broiler, Coccidiosis, Eimeria, Mekelle, Poultry, Prevalence*

CHAPTER I: INTRODUCTION

All domestic birds rose for human food production (meat and eggs) are considered poultry. The poultry industry is a rapidly growing sub-sector of agriculture that makes a substantial contribution to global nutrition and serves as a major driving force of the economy, greatly contributing to agricultural production through the supply of meat and eggs (Mottet & Tempio, 2017).

In Ethiopia, chickens are the common and almost the only poultry farmed for food production and other socioeconomic purposes. The majority of poultry operations in Ethiopia are backyard endeavors with a few low-yielding scavenger chickens. This method of producing eggs and meat is insufficient to meet the growing demand for these products in urban areas. Meat and egg prices have been rising steadily, which suggests that local demand is growing (Urgesa, 2023). The Ministry of Agriculture in Ethiopia has designated the production of chicken as a crucial priority area in its efforts to improve household food security and advance general well-being (Hailemichael *et al*., 2016). Nonetheless, infectious illnesses are common on commercial farms and cause large losses in terms of money (Asfaw *et al*., 2021).

Poultry production has been significantly impacted by various constraints, with poultry diseases playing a central role in hindering its development (FAO, 2008). In Ethiopia, prevalent diseases such as Newcastle disease, coccidiosis, salmonellosis, and chronic respiratory disease have hindered poultry production (Ame, 2023). Parasitic disease outbreaks pose a significant economic risk to commercial poultry farms and their integration, potentially leading to substantial employment loss (Abouelenien *et al*., 2021). Among parasitic diseases, Coccidiosis is particularly dangerous and commonly causes huge economic losses (Abdisa T *et al*., 2019). It is thought to be the source of 95.6% to 98.1% of the financial losses in the commercial broiler sector (Bera *et al*., 2010).

Coccidiosis is acknowledged as the primary parasitic disease of poultry and is caused by single-celled protozoan parasites of the genus *Eimeria* (Barnes & Gross, 1997; Mesa-Pineda *et al*., 2021). The disease significantly hinders the growth and feed utilization of infected birds, leading to a loss of productivity. This is due to the fact that *Eimeria species* affect the epithelial lining of the poultry intestine (Conway & McKenzie, 2007). The disease is important contributor to higher chicken mortality and illness globally, with varying clinical effects in different production systems. Persistent infections and prolonged untreated exposure in broiler production can lower feed conversion efficiency and growth rate, and in severe cases, cause intestinal tissue damage, increasing the risk of secondary infections with opportunistic pathogens (Yegani & Korver, 2008).

The coccidia oocyst, with its resilient wall, can withstand mechanical, chemical, and proteolytic damage. Its infectiousness is due to its sporulation, and clinical coccidiosis is influenced by host, agent, and environmental interactions (Mai *et al*., 2009; Lal *et al*., 2009). As the importance of poultry is a pivotal component of the food industry continues to grow rapidly, much has been done to optimize performance parameters and reduce the incidence of coccidiosis (Tellez *et al*., 2014). Disease control, especially in poultry, is crucial for minimizing costs related to animal losses and treatment, as well as reduced productivity. Key measures include maintaining high biosecurity and husbandry practices, and administering suitable vaccines and prophylactic treatments when appropriate. Biosecurity is particularly effective in controlling the introduction, establishment and spread of infectious agents within and among poultry flocks, farms, and countries (Graham *et al*., 2008).

Farm biosecurity encompasses all measures implemented to prevent the introduction and spread of infections on a farm, making it essential for protecting livestock against infectious diseases, particularly in conventional modern production systems (Dewulf & Immerseel, 2019). Biosecurity is primarily focused on creating a strong barrier between poultry and its environment, as well as the sources of infectious disease agents. A barrier that can keep pathogens from entering a protected population of birds should be established through a set of biosecurity measures.

There are various ways of categorizing biosecurity measures, all of which represent the fundamental principles of bioexclusion and biocontainment. The term bioexclusion refers to measures aimed at reducing the risk of introducing and spreading infectious agents into a new area, farm, or country. Biocontainment is another biosecurity measure used to prevent the release of pathogens from an infected herd (FAO, 2008).

Both the principles of bioexclusion and biocontainment involve isolating flocks through housing and building fences, controlling traffic to restrict the movement of products, livestock, and people, and maintaining sanitation through disinfection and cleanliness in flocks and the use of personal protective equipment (Conan *et al*., 2012). The science of biosecurity has been well established in the developed world while little attention has been made in developing countries such as Ethiopia. However, except few studies focusing on qualities assessment of biosecurity in Ethiopia, quantitative measurement of biosecurity scores in broiler farms has been lacking. Hence, it has been difficult to convince broiler producers, policy-makers, researchers and development partners to give due attention to the adoption good biosecurity practices in the country. To fill this evidence and knowledge gap, the current study was conducted with the main goal of quantifying and comprehending the relationship between the frequency of poultry coccidiosis and the level of biosecurity practices in a commercial broiler farms located in Mekelle city.

1.1. Statement of the problem

Coccidiosis is well known for being a horrible disease that causes financial loss when it comes to parasite diseases that affect the broiler farms (Fatoba & Adeleke, 2018a). It is predicted to cost around $11.93 billion annually because of higher mortality, the cost of treatment and vaccinations, and a decline in broiler performance as determined by feed intake, weight gain, and the feed conversion ratio (Blake *et al*., 2020). Poultry production is currently intensifying, increasing the likelihood of coming into direct touch with their excrement in a moist environment, which assures the survival of the oocysts and their transmission from flock to flock (Wondimu *et al*., 2019). The impact is worsened by various factors linked to poor health and management (Yohannes *et al*., 2014).

To control and prevent coccidiosis in broiler farms, this study aimed to quantify the relationship between poultry coccidiosis incidence and biosecurity practices on commercial broiler farms. A cross-sectional study conducted from January to November 2024 assessed biosecurity levels and coccidiosis prevalence in small and medium-sized broiler farms in Mekelle, Tigray. External and internal biosecurity measures including appropriate entry, feed supply, farm location, cleaning, disinfection, and farm hygiene lock can lower disease risk and antibiotic resistance are highly required (Tilli *et al*., 2022). However, empirical evidences which show the relationship between occurrences of coccidiosis and scores of biosecurity practices was unknown. Hence, no evidence was available that show whether coccidiosis is occurring in broiler farms with poor or good biosecurity scores or regardless of the biosecurity scores. More importantly, no quantitative evidence was available which show the external and internal biosecurity scores of the broiler farms. Hence, the biosecurity scores of broiler farms in Mekelle were not known as comparing country and global scores. As a result of which, despite the fact that biosecurity should be the basis for disease prevention and control efforts, it was not adequately included in the poultry diseases prevention and control strategies, Ethiopia.

A major information gap was highlighted by the fact that most farm owners are unaware of the disease and biosecurity precautions. Additionally, the agricultural industry and individual farms are at significant danger due to the paucity of research on broiler poultry biosecurity procedures. Many farmers could undervalue the significance of strict biosecurity measures, leaving them susceptible to disease. Biosecurity, which includes procedures and policies that reduce transmission, improve flock health, and safeguard productivity, is crucial to stopping the spread of illness in chicken farming. But because the biosecurity system at the research site was not quantified, this study aimed at filling the evidence gaps and offer suggestions for enhancements. To achieve this, occurrence coccidiosis was considered as indicator to quantitatively measure the biosecurity practices of broiler farms in Mekelle.

1.2. Objective of the Study

1.2.1. General objective

The general objective of this study was to quantify Biosecurity scores and prevalence of coccidiosis in small and medium scales commercial broiler poultry farms in Mekelle city.

1.2.2. Specific Objectives

- To determine the prevalence of coccidiosis in broiler poultry farms
- To determine the Biosecurity scores in commercial broiler poultry farms
- To identify risk factors that contribute to the association of biosecurity measures and occurrence of coccidiosis
- To characterize gross lesion of due to coccidiosis by postmortem examination

1.3. Significance of the Study

Poultry coccidiosis, caused by protozoan parasites of the genus Eimeria, poses significant health risks to chickens due to its widespread occurrence in Mekelle. Quantifying the prevalence of the disease and biosecurity scores of broiler farms were essential for effective management, prevention, and treatment strategies. This study provided valuable evidences and insights for poultry producers, and various stakeholders, including the private sector, veterinarians working for private, in governmental and non-governmental organizations, and researchers, so as to improve broiler health and productivity. Furthermore, it may serve as a reference for researchers to incorporate biosecurity as important disease prevention and control strategies in poultry farms.

CHAPTER II: LITERATURE REVIEW

2.1. Coccidiosis

Many domestic animals are at risk of coccidiosis, including poultry, rabbits, ruminants, and carnivores. Swine, however, are less susceptible to this disease (Lilić *et al.*, 2009). Coccidiosis, which mainly affects chickens, is caused by 9 species of the Eimeria parasite. It is the most widespread parasitic disease, leading to around $3 billion in annual economic losses globally (Mohammed & Sunday, 2015; Latif *et al*., 2016). In Ethiopia, avian coccidiosis, having a country level pooled prevalence of 37%, is among the top five poultry diseases causing huge economic losses (Yohannes *et al*., 2019).

2.2. Etiology

Coccidiosis occurs when sporulated oocysts are introduced to chickens. An oocyst is a durable structure formed by enclosing the parasites' soft bodies in a glycoprotein and lipid bilayer wall (oocyst wall), enabling it to endure in the external environment for an extended period, even in harsh conditions (Mai *et al*., 2009; Remmal *et al*., 2011). There are two kinds of oocysts based on their infectious capacity, according to Lal *et al.* (2009): non-infective, unsporulated oocysts, and infectious, sporulated oocysts. While unsporulated oocysts remain in the host caecum for 7 months, sporulated oocysts can endure for around 50 months in the external environment (feces, litter, feed, and soil) before entering a host (Fatoba & Adeleke, 2018).

As a group, coccidia of the genus Eimeria (Eimeridae family) are species-specific, infecting a single host species or a group of closely related hosts (Müller & Hemphill, 2013). It is characterized by obligate intracellular parasites, which possess unique specialized organelles that form the apical complex. These include: micronemes, rhoptries, dense granules, and conoid and polar rings that provide the structural stability required during invasion of the host cell (Suarez *et al*., 2017). Despite the fact that nine different species of Eimeria have been linked to poultry coccidiosis, only seven of these species have been identified as pathogenic (Kahn, 2005).

2.3. Life cycle

An organism's life cycle is characterized by a series of abrupt developmental (maturation) changes in its morphology and/or ecology (Benesh, 2016). A direct life cycle comprising three developmental phases is exhibited by the protozoan genus Eimeria: schizogony (merogony), gametogony (gamete creation for sexual reproduction), and sporogony. As to McDougald *et al.* (2020), these phases encompass both asexual and sexual reproduction. Furthermore, schizogony and gametogony are endogenous developmental phases, but sporogony is an exogenous developmental stage (López-Osorio *et al*., 2020).

Transmission occurs through the fecal-oral route, and infection starts with the ingestion of sporulated oocysts containing eight sporozoites, initiating the stage known as schizogony (Haug *et al*., 2008). The oocyst wall's structure and permeability are changed by the gizzard's mechanical activity and the enzymatic milieu of the digestive tract (Chapman, 1978). The Stieda body, which is a protein and carbohydrate plug found at the sporocyst's pointed, narrow end, is being removed by the sporozoites inside each sporocyst, allowing the sporozoites to escape into the oocyst cavity. They are released into the intestinal lumen via the oocyst micropyle during the process called excystation (Kheysin, 2013).

The parasitic feeding phase, which lasts around 12-48 hours, is initiated when the sporozoites infiltrate the enterocytes and transform into trophozoites (Rose *et al*., 1984; Trout & Lillehoj, 1995). The trophozoite starts to grow, the parasitophorous vacuole forms, and the parasite nucleus divides asexually several times (Tierney & Mulcahy, 2003), forming the schizont or meront, which is full of merozoites. About 3 days after infection, the mature schizont bursts, releasing spindle-shaped merozoites. These merozoites carry an apical complex which aids in their movement and penetration of intestinal epithelial cells, starting the formation of new schizont generations that replicate through asexual means (Nabian *et al*., 2018). It is believed that the number of asexual reproductive phases is genetically predetermined and unique to each Eimeria species (Ahmad *et al*., 2016). This phase's primary goal is to increase the host's merozoite count in order to get ready for the sexual reproduction phase, which is a crucial part of every apicomplexan life cycle (Walker *et al*., 2013).

The stage of sexual reproduction known as gametogony begins after the asexual reproduction phase is finished. This stage consists of three phases: gametocytogenesis, where gametocytes are created from merozoites; gametogenesis, where haploid micro and macrogametes are differentiated from the gametocytes; and finally, fertilization of macrogametocytes by microgametocytes, which results in diploid zygotes, which marks the completion of sexual reproduction. At this stage, meiosis happens inside the protective oocyst wall, and is followed by mitosis to produce the infectious sporozoites (Kheysin, 2013; Walker *et al*., 2013).

The third step of the cycle, sporulation, takes place when the animal excretes the oocyst in its feces (You, 2014). If the environmental conditions are suitable, the diploid oocyst begins sporogony formation, which happens in three stages (Kheysin, 2013): At first stage the zygote nucleus divides, and the cytoplasm is ready and rearranged. Four nuclei are created when this division is done twice. Second stage four sporoblasts form, undergo cytoplasmic restructuring, pass through the pyramidal stage, and become oval sporoblasts, which will eventually result in the creation of four sporocysts. At this point, there is no nuclear division. At last formation of sporozoites, every sporocyst undergoes a single nuclear division, and the cytoplasm splits into two longitudinal sections to create two sporozoites with a Stieda body at the end of each sporocyst (Müller & Hemphill, 2013). The ideal circumstances of oxygen, temperature, and humidity are necessary for this process to take place (Waldenstedt *et al*., 2001).

2.4. Epidemiology

The study of coccidiosis epidemiology is a timely matter that should be established in order to identify the species and potential risk factors that cause the diseases, as well as to design a preventive production system, agro-ecology, and a level of control regimen that is appropriate for the local management. It is not possible to create a coccidia-free environment in farming conditions (Jordan *et al*., 2002). Due to favorable ecological and managerial conditions that allow the causal agent to flourish and spread year-round, the disease is endemic in the majority of tropical and subtropical locations (Obasi *et al*.,

2006). There are significant regional and worldwide variations in the prevalence of certain combinations of Eimeria species and the severity of illness (Haug *et al*., 2008).

Coccidia oocysts sporulate under warmth condition of about 25–30 ℃ with adequate aeration and water while dry condition at 10 ℃ delays sporulation (Mohammed & Sunday, 2015). According to Musa *et al.* (2010), the illness damages the intestine and the caecum, and it takes 5-7 days for an outbreak of Coccidia infection to begin when chicks consume large amounts of sporulated oocyst. A study on the spatial distribution of research on infectious and parasitic diseases in poultry in Ethiopia reveals a concentration in central Ethiopia, particularly in the Oromia region, with minimal research conducted in Tigray, accounting for only 5.45% (6/110) (Asfaw *et al*., 2019). Ayane & Berhanu, (2020) reported a 19.4% prevalence of poultry coccidiosis in Mekelle, underscoring its significance as a major poultry disease.

2.4.1. Agent factors

Coccidial infections are self-limiting and mostly determined by the quantity of swallowed sporulated oocysts (McDougald *et al*., 2020). The parasite's short prepatent phase and strong biotic potential cause the quantity of oocysts in the litter to increase quickly (Jordan *et al*., 2002). Extremely high dosages of consumption have the potential to induce the "crowding effect," which stops the parasite's life cycle from continuing while still causing intestinal damage (Jenkins *et al*., 2017). For example, Williams (2001) was able to characterize the reproductive potential of each species of *Eimeria* under experimental conditions, using infective doses of 903, 16, 39, 14,16, 16, or 72 sporulated oocysts, of *Eimeria acervulina, Eimeria brunetti, Eimeria maxima, Eimeria mitis, Eimeria necatrix, Eimeria praecox or Eimeria tenella,* respectively.

2.4.2. Host factors

In chickens, the host plays a crucial role in Eimeria infections (McDougald *et al*., 2020). Certain homologous parasites can induce protective immunity against a future challenge by the same parasite because some Eimerian parasites are particularly immunogenic in hens (Lillehoj & Lillehoj, 2000). Birds that have never encountered coccidiosis before

can get the disease at any time. However, it mainly impacts young birds. Due to the time it takes for coccidia populations to reach harmful levels, outbreaks usually occur when birds are 3–8 weeks old (Fanatico, 2006).

2.4.3. Environmental and management factors

Certain circumstances put the parasite in danger and accelerate its spread, such as weak biosecurity procedures and unhygienic treatment of staff and equipment (Wondimu *et al*., 2019; Morishita & Greenacre, 2021). Sanitization is crucial in mitigating the spread of the parasite (Peek, 2010), since the most common means of oocyst transmission is by mechanical vectors, like humans or equipment moving between farms, as well as by the presence of rodents and insects, like flies and beetles (McDougald *et al*., 2020). The study conducted by Hadipour *et al*. (2011) found that the prevalence was not affected by flock size, but it was affected by management. Inadequate management practices, such as using wet litter that promotes oocyst sporulation, contaminating drinkers and feeders, providing inadequate ventilation, and using high stocking density, can exacerbate clinical signs.

2.5. Pathogenesis

The oral intake of sporulated oocysts through contaminated feed and/or water is the route of infection. Following ingestion, infectious oocysts expel the infectious form known as the sporozoite. Intraepithelial lymphocytes assist in the transfer of the sporozoites up to the major lesion locus. In the intestines, the protozoan parasite of the genus Eimeria grows and damages tissue, impairing nutrition absorption, causing dehydration, blood loss, skin pigmentation loss, and making the body more vulnerable to other disease-causing infections (McDonald & Shirley, 2009). Additionally conditions that weaken a bird's immune system can combine with coccidiosis to cause a more serious issue, including For instance, infectious bursal disease can worsen a coccidia infection, while Marek's illness can prevent the establishment of coccidiosis immunity (Boulianne *et al*., 2020).

Pathogenesis of the infection is affected by species of the eimeria, concurrent illness and nutritional variables. The most harmful Eimeria species in chickens are Eimeria necatrix and Eimeria tenella, as they cause severe bleeding when they generate schizogony in the lamina propria and crypts of the small intestine and ceca, respectively. Though Eimeria brunetti is rare but pathogenic when it does appear, Eimeria arcevulina, Eimeria maxima, and Eimeria mitis are common and slightly to moderately pathogenic. Particularly non-pathogenic species include, Eimeria mivati Eimeria praecox, and Eimeria hagani (Haug *et al*., 2007).

2.6. Clinical Sign

Chicken coccidiosis can arise in two forms: subclinical coccidiosis and clinical coccidiosis. Sub-clinical coccidiosis is characterized by worse feed efficiency, slower growth, and eventually greater rates of morbidity and mortality (Dakpogan & Salifou, 2013; Abdisa T *et al*., 2019). Chickens suffering from clinical coccidiosis exhibit drooping eyelids, ruffled feathers, pale comb, wattles, and internal organs (caused by blood loss), smaller heads, discolored beaks and shanks, catarrhal inflammation, dehydration, and hemorrhagic feces (Abebe & Gugsa, 2018).

When eimeria species enter the intestinal mucosa, they cause some inflammation and damage to the epithelial cells. In a short period of time, meronts, gamonts, and oocysts produce significant histological changes to the host intestinal epithelial cells, including deformation, rupture, separation from neighboring cells, and sloughing (Yun *et al*., 2000). Infected birds display ruffled feathers, signs of depression or drowsiness. Additionally, their feed and water intake decrease, and their feces may become watery, whitish, and occasionally bloody (Yohannes *et al*., 2014). This can lead to dehydration, hindered weight gain, and if untreated can result in death. Moreover, malabsorption happens due to decreased brush border enzyme function and disruption of intestinal integrity (Morishita & Greenacre, 2021). Infection can also produce other intestinal changes; for example, inoculation with *Eimeria acervulina* and *Eimeria maxima* oocysts increased the size and quantity of goblet cells along illeal crypts in broiler (Collier *et al.*, 2008).

Goblet cells are key defense mechanisms in the intestinal tract because they secrete glycoproteins of large molecular weight called mucins (Al-Quraishy *et al*., 2020). Mucins are the initial line of defense against intestinal pathogens, and they protect the epithelium from pathogens and irritants in the intestinal lumen (Montagne *et al*., 2004). Similarly, it has been shown that when *Eimeria tenella* invades cecal epithelial cells, the cecum increases the rate of mucus production and promotes a protective phenotype as an immune response to the parasite (Mesa-Pineda *et al.*, 2021). But there is a risk associated with this increased mucin production, since it might encourage secondary colonization by other diseases (Collier *et al*., 2008; Adhikari *et al*., 2020). This further impacts intestinal health by hindering metabolism and nutrient absorption (Khater *et al*., 2020).

2.7. Diagnosis

For the purpose of diagnosis and disease management, it is crucial to correctly identify the species of Eimeria (Carvalho *et al*., 2011) and, in terms of business, a coccidiosis diagnosis is necessary when the lesions are noticeable (McDougald *et al*., 2020; Conway & McKenzie, 2007). The assessment of Eimeria infections is traditionally done in two ways: under a microscope, where the size and form of oocysts is assessed; under a macroscopic diagnosis, clinical signs in infected animals are observed, along with the location and appearance of gross lesions during necropsy (Conway & McKenzie, 2007). It is occasionally also incorporated to assess other developmental stages in microscopic smears (Barrios *et al*., 2017). Molecular diagnostics can also be used in cases when higher diagnostic precision is required (Hauck *et al*., 2019; Hinsu *et al*., 2018).

2.7.1. Postmortem examination

The type and locations of the intestinal lesions can be used to identify the species of the genus Eimeria (Carvalho *et al.*, 2011). The degree of inflammation and damage to the intestinal tract determines the coccidiosis lesions. These include mucoid to blood-tinged exudates, petechial hemorrhages, necrosis, thick intestinal wall, hemorrhagic enteritis, and copious bleeding of mucous in the ceca (Gazoni *et al*., 2020). On histopathology, the gut wall is thickened, indicating fluid retention.

There could be blood in the gut lumen, which would indicate bleeding or just an excessive amount of blood being retained in the tissue (hyperemia). Additionally, infiltration causes a range of physiological effects as well as the emergence of an immunological response (Marquardt *et al*., 2000). A standardized scoring technique is used for evaluating gross lesions in the intestines, where a score from zero to four is assigned. The aim is to numerically classify the gross lesions caused by each Eimeria species (Barrios *et al*., 2017; Khater *et al.*, 2020). Starting with the duodenum, the entire bird's gut must be assessed for this score system. A good light source (solar or lamp) is necessary for accurate scoring while examining the mucus and serous membranes to detect lesions (Conway & McKenzie, 2007).

Table 1. Characteristic lesions of Eimeria infection during post mortem examination

Eimeria species	**Location**	**Lesion**
Eimeria necatrix	Middle intestine	Severe hemorrhage with mucoid discharge whitish and red spot in wall of intestine
Eimeria maxima	Middle intestine	Distended intestine with hemorrhage spots, mucoid discharge
Eimeria brunetti	Lower half of Intestine	Thin walled intestine, mucoid on necrotic discharge, distension of intestine
Eimeria tenella	Ceca	Severe hemorrhage with white red spots in wall of intestine
Eimeria acervulina	Upper intestine	Whitish spots on wall on serous surface hemorrhage streak and whitish lesions on intestinal surface, mucoid enteritis
Eimeria praecox	Duodenum	No lesion but slightly hemorrhagic appearance on intestinal surface of duodenum slight mucoid discharge.

Adapted from Abebe and Gugsa, 2018

2.7.2. Fecal Microscopic Examination

The most practical and affordable approach of detecting coccidiosis in hens is by faecal examination methods, which detect Eimeria oocysts discharged through the faeces of sick chicken (Mwale & Masika, 2011). The two most frequent qualitative faecal examination diagnosis procedures for coccidiosis in chickens are microscopic viewing of oocysts in smears prepared from emulsified faeces or checking the concentration/amount of floated oocysts by floatation technique (Alqomsan, 2010). The relatively low specific gravity (1.05–1.15) oocysts are brought to the surface by a solution with a higher specific gravity (saturated glucose, 1.44; saturated sodium chloride, 1.19), which leaves other waste materials in the feces at the bottom of the solution (Olanrewaju & Agbor, 2014).

A quantitative fecal analysis is performed to determine the OPG count (oocysts per gram of feces), oocyst sizes, and the proportion of sporulation (Singla & Gupta, 2012). The most popular laboratory analytical approach for figuring out the individual oocyst shedding pattern of an infected bird and counting Eimeria oocysts in the faecal solution is the McMaster method and the hemocytometer method (Conway & McKenzie, 2007). The oocyst flotation method is the same as the quantitative methodology. It takes place in a special counting chamber/slide known as the McMaster slide, which facilitates counting with a light microscope (Vadlejch *et al*., 2011). Still, the McMaster approach requires a higher degree of expert knowledge to discriminate between Eimeria species based on their morphology, and the OPG count does not go into detail on how the Eimeria parasite negatively impacts flock performance (Gussem, 2007).

2.7.3. Molecular Diagnosis

In real-world settings, Eimeria infections are frequently caused by many species that share similar clinical traits, which makes field diagnosis challenging (Carvalho *et al*., 2011). This indicates that for an accurate diagnosis, more objective and sensitive methods are necessary (You, 2014).

Polymerase chain reaction (PCR) is a highly sensitive molecular diagnostic method used to detect Eimeria species in chickens by analyzing variations in their genomic DNA

(Alqomsan, 2010). Through an enzymatic reaction, the polymerase chain reaction (PCR) is a basic technique that amplifies particular areas of deoxyribonucleic acid (DNA). Template DNA, primers, thermostable DNA polymerase, deoxynucleotide triphosphates (dNTPs), and a buffer containing potassium and magnesium are the five essential ingredients needed for PCR amplification (Lorenz, 2012). The use of strict cycling conditions and sequence-specific primers in the reaction are major contributors to the high specificity of PCR. PCR programs follow the same three basic steps of denaturation: heating to separate the DNA molecule's double strands; annealing to enable primers to attach to their complementary target sequence; and extension, which involves heating the sample to a temperature marginally higher than annealing that is ideal for DNA polymerase to synthesize the new double-stranded molecule (Holland *et al*., 1991).

2.7.4. Biochemical Diagnostic Method

In order to prevent and control coccidiosis, next-generation laboratory diagnostic techniques are essential in place of the previously described traditional techniques for determining the precise species of Eimeria that is causing the infection (Fatoba & Adeleke, 2018). Additionally, by using laboratory-developed diagnostic techniques that are species-specific and have a better rate of sensitivity and accuracy, the shortcomings of conventional approaches can be solved(Singla & Gupta, 2012). An easy-to-use and trustworthy biochemical diagnostic technique for coccidiosis is the enzyme-linked immunosorbent assay (ELISA), which finds Eimeria species-specific epitope-specific antibodies (IgG and IgM) in serum (Constantinoiu *et al*., 2007).

2.8. Treatment

Since 1948, when sulphaquinoxaline and nitrofurazone were initially licensed by the American Food and Drug Administration, a number of anticoccidial medications have been introduced and have significantly contributed to the expansion of the poultry industry (Conway & McKenzie, 2007). Across the world, chemoprophylaxis has been implemented as a successful model for coccidiosis prevention.

It involves adding a variety of anti-coccidial medications to drinking water or poultry diets, which have the dual effects of coccidiocidal (which destroys Eimeria species) and coccidiostats (which stop replication and growth) (Quiroz-Castañeda & Dantán-González, 2015). Most broad-spectrum anti-coccidial drugs mainly target the asexual phases (first and second), with some also affecting the sexual phases of the Eimeria life cycle, and a few drugs disrupt the metabolic pathways of Eimeria species (Kant *et al*., 2013).

The anticoccidial products can be divided into three categories based on where they came from: mixed products, synthetic chemicals, and polyether antibiotics or ionophores. Synthetic compounds are created through chemical synthesis and commonly known as 'chemicals'. These drugs target the parasite metabolism in a specific way. For example, amprolium and the parasite compete for thiamine (vitamin B1) absorption. Ionophores are produced by fermenting Streptomyces or Actinomadura species and work by disrupting the balance of crucial ions such as sodium and potassium to destroy coccidian. A few medication mixes that contain two synthetic compounds or a synthetic chemical and an ionophore are combined to create mixed compounds, which are also used to treat coccidiosis (Allen & Fetterer, 2002).

2.9. Control and Prevention

Prevention of coccidiosis can be achieved much easier than treatment and, due to its drastic effect on poultry; different control methods have been deployed. The main strategies used to prevent and control coccidiosis are vaccinations, natural feed additives, prophylactic anticoccidial drugs, and improved handling practices on farms. Maintaining litter conditions that minimize oocyst sporulation can be beneficially achieved through the use of clean water, adequate air, and facility cleaning and disinfection (Fatoba & Adeleke, 2018).

Preventive medications for coccidiosis, known as coccidiostats, must effectively inhibit the schizogonic stage and allow for immunity to develop. Early prophylactic use is essential as much of the damage occurs prior to symptom onset, and medications are

unable to completely stop an outbreak (Kahn, 2005). When chickens are infected with a small number of Eimeria parasites, they develop protective immunity after two or three consecutive infections (Del Cacho *et al*., 2016).

2.9.1. Control with Anticoccidial Agents

Anticoccidials can be classified into coccidicides and coccidiostats according to how they work. Coccidiostats prevent the parasite from developing, hindering its growth and replication; nevertheless, their effects are reversible, as the disease may resurface if they are removed from the diet. The characteristic of a coccidicide is that it kills the parasite or damages it irreversibly (Peek, 2010).

The prolonged use of these anticoccidials as a preventative measure has caused the parasite to become more resistant, which has reduced the compounds' effectiveness (Khater *et al*., 2020). In order to combat this, anticoccidials are currently utilized in dual (or shuttle) or straight rotation programs. In the first, two or more anticoccidials, typically with different modes of action, are alternated in the various feeds provided during the life cycle of the chicken; in the second, the same medication is used continuously during a production cycle but is substituted with a different medication after one or more flocks (Quiroz-Castañeda & Dantán-González, 2015; Peek & Landman, 2011).

2.9.2. Vaccination

The public's concern over chemical residues in poultry products, the development of resistance to anticoccidial drug use, and environmental pollution has sparked research into alternative control strategies like early vaccination or drug development (Yim *et al*., 2011).

Chickens can develop active or passive immunity against poultry coccidiosis to reduce its harmful effects (Lee *et al.*, 2009; Wallach, 2010). Attenuated and virulent vaccines are the sorts of vaccines that are currently available for immunizing hens (Chapman & Jeffers, 2014). Because attenuated vaccines lack a portion of the parent strain's life cycle (fewer asexual reproductive cycles), their potential for reproduction and pathogenicity is reduced (Mathis *et al*., 2018). Attenuation aims to lessen the parasite's pathogenicity and,

thus, its harmful effects on the host. Numerous techniques for attenuation have been employed, such as repeated passage in chicken embryos, chemical therapy, radiation (Fetterer *et al*., 2014), and precociousness selection (Jeffers, 1975) Precocious lines of Eimeria are distinguished by a reduced endogenous life cycle as a result of the eradication of one or more schizogonies, which lessens intestinal tract damage and oocyst generation. Reproductive potential and pre-patent time of the selected Eimeria species are reduced by selecting the first oocysts discharged in the feces to inoculate hens at the second pass and repeatedly repeating this process. Pathogenicity also decreases concurrently with these reductions, but immunogenicity is maintained (Fang *et al*., 2023). This is a key benefit for improving the effectiveness of virulent coccidial vaccines; however, due to the reduced reproductive capacity of attenuated vaccines, production costs are notably increased. The virulent vaccines are composed of anticoccidial-sensitive strains, while others are made of strains that are more or less resistant. The primary advantage of live anticoccidial-sensitive strain vaccines is their capability to modify the level of resistance within a specific coccidial population (Mathis *et al*., 2018).

Despite the cost and additional workforce, vaccination has long been recognized as the most effective strategy for controlling coccidiosis in chickens. This can be achieved through various methods such as spraying the vaccine on feed, mixing it with drinking water, using the spray cabinet method, utilizing vaccine incorporated edible gel pucks, and employing in-ovo injection methods (Fanatico, 2006; Sokale *et al*., 2017; Albanese *et al*., 2018).

2.9.3. Selection of genetically resistant chickens

Breeding chickens to be genetically resistant to coccidiosis is one possible control strategy (Zou *et al*., 2019). Disease control through host natural resistance is becoming an appealing alternative approach. This method has not been extensively explored, partly because of the effectiveness and accessibility of drugs, and partly due to the primary focus on breeding programs. Nevertheless, the interest in this strategy is growing with advancements in genetic manipulation technologies (Sid & Schusser, 2018).

2.9.4. Natural feed additives

Many natural items or feedstuffs have been investigated for use as dietary supplements with anticoccidial properties. Plant extracts high in antioxidants have been suggested to be beneficial in the treatment of coccidial infections (Allen & Fetterer, 2002b). There have been significant achievements in feeding chickens oregano extract or essential oil. Studies show that oregano essential oil can combat Eimeria tenella. Chickens treated with it displayed comparable body weight gains and feed conversion ratios to the uninfected control group. Nonetheless, one study proposed that oregano might solely work effectively in non-vaccinated birds against coccidian (Batungbacal *et al*., 2007).

Adamu & Chaiwat, (2013) noted the protective impact of Moringa stenopetala leaf-enriched diets on Eimeria tenella-infected broiler chickens. Their study revealed that a diet containing Moringa stenopetala leaf powder notably decreased the oocyst count in infected chickens, comparable to those given amprolium, when compared to the control group. Moreover, the inclusion of Moringa leaf powder in the diet also lessened the cecal lesion scores in eimeria tenella-infected chickens, similar to the effect of amprolium supplementation.

2.10. Biosecurity in Poultry Production

Medication/vaccination, biosecurity, and effective farm management are the three facets of the disease control triangle. Biosecurity is a cost-effective and effective disease control program for farm operations, managing broiler health and minimizing disease risks, especially for economically sensitive diseases (Sharma *et al*., 2018). The notion of biosecurity is strategic and integrated, encompassing the regulatory and policy frameworks (including tools and activities) that analyze and manage risk in public health, animal and plant health, food safety, and related environmental risk (FAO, 2007). Moreover, Ferit Can (2018) defines biosecurity as all-encompassing infection control methods that consider sociocultural traits and attitudes, geographic, climatic, and epidemiological conditions, as well as educational attainment. One of the most important concerns facing developed, developing, and transitional nations is biosecurity. Research and practice have shown that, despite the acknowledged significance of biosecurity, there

are still significant gaps in the implementation of preventive measures on chicken farms (Van Steenwinkel *et al*., 2011). Furthermore, the basis for the biosecurity of the entire production chain is provided by biosecurity at the farm level (Siekkinen *et al*., 2012).

Biosecurity encompasses three main types: isolation to protect chickens from infections, traffic control to limit movement, and sanitation control to clean and restrict equipment movement (Arabi & Gumaa, 2021). The poultry industry's susceptibility to disease necessitates strong biosecurity measures on farms to safeguard against both deliberate and accidental biological threats (Ali *et al*., 2014). A poultry farm's biosecurity encompasses all steps taken to reduce the possibility of disease agents being introduced and spreading, which in turn includes all efforts to maintain the health of the farm and its chickens. On-farm animals are shielded from endemic and epidemic diseases by implementing these biosecurity measures and managing them effectively (Dewulf & Van Immerseel, 2019).

Biosecurity is divided into external and internal components. External biosecurity aims to prevent diseases from entering or leaving the farm, focusing on interactions with the external environment. This includes both endemic diseases, which are common in a country but not present on every farm, and exotic diseases, which are rare in the country (Ribbens *et al*., 2008). Taking all required precautions to prevent the spread of illnesses within the farm is known as internal biosecurity (Laanen *et al*., 2013). Others claimed that the conceptual, structural, and operational frameworks of biosecurity measures include the design and construction of housing as well as management practices that prevent infectious diseases from infecting the flock (Scott *et al*., 2018; Maduka *et al*., 2016).

Limiting the amount of infections at the farm is the primary goal of biosecurity. In this approach, an animal's immune system will be less stressed, which lowers the danger of illness outbreak and, ultimately, improves the health and welfare of the animal. Implementing a segregation, hygiene, or management procedure (apart from medically effective feed additives and preventive/curative animal treatment) with the specific goal of lowering the likelihood that any potential pathogen will be introduced, established, survive, or spread to, within, or from a farm, operation, or geographic area is known as a

biosecurity measure (Huber *et al*., 2022). The selection of farm facilities' locations should be the first step in implementing biosecurity management. Physical aspects of farms, such as layout, drainage, and fence, should come in second, followed by standard practices like bioexclusion and infection spread (biocontainment) within a facility. Such activities and procedures should be routinely evaluated and practiced by any disease control program (Tsegaye *et al.*, 2023).

By lowering the possible dangers of a disease outbreak through the use of biosecurity measures, the farm may also experience other positive outcomes. In the context of disease transmission, not every channel of transmission is equally significant. Ranking the various routes based on their relevance is so difficult. This is mostly because infectious agents differ greatly in their capacity to infect living things, including their odds of surviving in the environment. It follows that different biosecurity measures will have varying effects on preventing various infectious diseases that affect chicken (Gelaude *et al*., 2014; Amalraj *et al*., 2024).

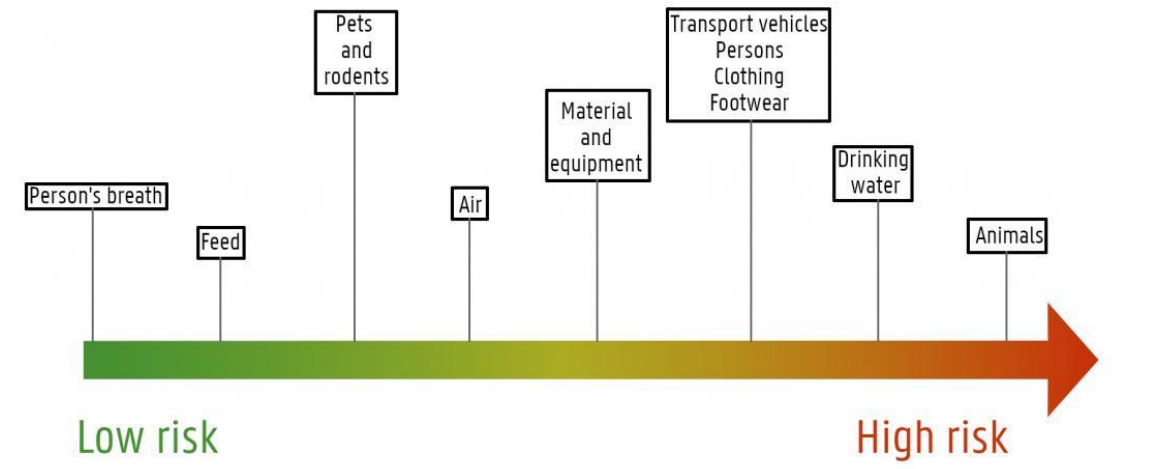

Figure 1. Disease transmission route and their level of risk

Adopted from Laanen *et al*. (2013)

The ubiquity, greater capacity for dissemination, and increased capacity for reproduction of coccidia oocysts make it difficult to maintain hens free from coccidiosis (P. C. Allen & Fetterer, 2002a). Thus, the goal of all management and biosecurity procedures should be to stop coccidia parasites from entering the farm and/or, in the case that an infection has

already occurred, to stop the parasites from multiplying and from spreading the illness (Peek & Landman, 2011). In order to control coccidiosis, the farm premises must be thoroughly cleaned and disinfected. Many disinfectants have been identified and used worldwide for the destruction of sporulated and non-sporulated oocysts. These substances include ammonium hydroxide (in both liquid and vapor forms at concentrations of (≥5%), cresol-based products, a mixture of formol (37%) and sodium dodecylbenzene sulphonate (12%), and a blend of calcium hydroxide and ammonium sulfate (Peek & Landman, 2011). Because excreted oocysts can spread coccidiosis, the litter should be kept dry at all times, raked often, and replaced every two flocks to prevent oocyst sporulation. In addition, lime powder can be used as a litter drying agent during rainy seasons (Tellez *et al*., 2014; Abdisa *et al*., 2019).

Moreover, coccidiosis outbreaks in broiler farms can be generally avoided by separating the chickens from the outside world, minimizing dangerous movements within and between farms, sanitizing people and equipment, providing adequate ventilation, reducing the number of birds per acre, and supplementing clean feed and drinking water (Peek & Landman, 2011; Quiroz-Castañeda & Dantán-González, 2015).

Biosecurity is essential for reducing the spread of infectious diseases, requiring a shift in conventional poultry farming practices (Sharma *et al*., 2018). The biosecurity status of chicken farms in Ethiopia requires enhanced measures through training and awareness for farm owners and employees, as small and medium-scale farms exhibit inadequate biosecurity (Ismael *et al*., 2021). Implementing effective biosecurity measures can enhance flock health, reduce treatment costs, minimize losses, increase farm profitability, protect against disease, and facilitate disease management if it occurs (Goualie *et al*., 2020). The Food and Agriculture Organization (FAO) strongly supports the strict implementation of biosecurity measures as the most effective way to prevent and manage the spread of contagious diseases (Tasie *et al*., 2020).

CHAPTER III: MATERIALS AND METHODS

3.1. Study Area

The research was conducted in Mekelle City from January to November 2024. Mekelle city, the capital of Tigray regional state, is situated between 13º 09" and 14º34" North latitude and 39º 12" and 40º28" East longitude. The city is approximately 784 kilometers north of Addis Ababa and has a midland climate, locally known as Weyna dega, with an elevation of 2,254 meters above sea level. The average annual temperature ranges from 11.11℃ to 24.1℃, and the estimated annual rainfall average is 570 mm (Kiros *et al*., 2024).

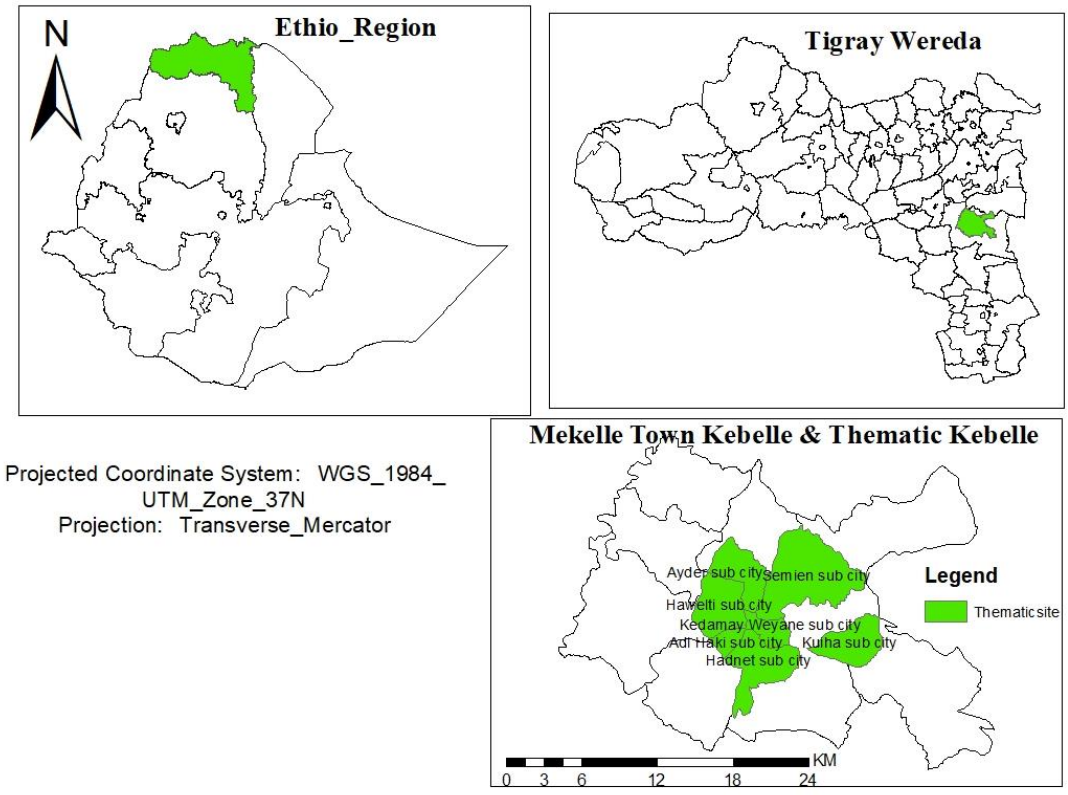

Figure 2. Map of the study area

3.2. Study Animals and Farms

The study animals were from broiler chicken of 23 small scales (with 100-1000 birds) and 15 medium scales (with 1001-5000 birds) broiler farms (Urgesa, 2023). According to the information gained from the Mekelle city Administration agricultural coordinator, the total number of medium and small-scale intensive poultry producers (farms) was estimated to about 300 with total population of 122,656 chickens. It is crucial to remember, too, that the number of farms in operation varies significantly.

3.3. Sampling procedure and sample size determination

To quantify the farm biosecurity practices and determine the prevalence of chicken coccidiosis, a cross-sectional study design was carried out.

3.3.1. Sampling procedure

Random sampling was employed to collect chicken droppings and farms were purposefully included in the study based on availability, owner willingness, and adherence to the inclusion criteria to determine biosecurity and prevalence of coccidian infections. To achieve this, data on the total number of poultry farms was obtained from Mekelle Agriculture office. However, purposive sampling strategy was used for postmortem examinations of dead/severely sick chickens.

3.3.2. Sample Size

A total of 257 chicken dropping samples were collected from 38 small and medium scale broiler farms to determine the prevalence of coccidiosis and associated factors through parasitological examination and all of them were assessed for biosecurity measure. The sample size was determined using the formula recommended by (Cochran, 1963), based on the previous prevalence result from a study conducted by Yohannes *et al*. (2014), which reported a clinical coccidiosis of 17.5% prevalence with a 95% confidence interval and a desired absolute precision level of 5%.

Using the formula $N = \frac{1.96^2 P\ exp(1 - P\ exp)}{d^2}$

Where,

n = the required sample size,

p = the expected prevalence, and

d = the standard error

Thus, 222 samples was the minimal sample size required for the fecal inspection in this investigation. The sample size was expanded to 257 in order to improve precision.

3.3.3. Data collection procedure

3.3.3.1. Chicken droppings examination

To gather data, chicken dropping samples were collected using sterile containers that had been clearly labeled. Each sample was recorded using a standardized collection format that captured important details, including the type of flooring in the poultry housing, the age of the birds, the size of the flock, and the sex of the chickens. Age was categorized as either greater than eight weeks or less than or equal to eight weeks (Oljira *et al*., 2012). Fecal samples were collected from chicken immediately after excretion and transported to the Mekelle University College of Veterinary Medicine parasitological laboratory using an ice box. The samples were stored in a refrigerator at 4°C until analysis commenced. For the detection of oocysts, the flotation method was employed using a saturated sodium chloride solution, while the McMaster chamber method was utilized for counting oocysts per gram (OPG) (Long *et al*., 1976).

3.3.3.2.. Postmortem Examination

From 7 farms reporting disease outbreaks, 18 chickens exhibiting suspected coccidiosis symptoms (diarrhea, lethargy, poor body condition, in appetence, and ruffled feathers), and recently dead birds, were purposely sampled for a postmortem examination to identify gross lesions associated with the disease. The method Jacobs *et al*. (2019)

outlined for cervical dislocation was used to sacrifice the chickens. For observation of lesions the entire intestine was extracted from the bird without any damage. The gut was slit open, starting with the duodenum, and lesions were checked on the mucosal surface as well as the unopened serosal surfaces as described by (Conway & McKenzie, 2007).

3.4. Assessment of Biosecurity Measures

To ascertain the chicken farms' biosecurity protocols, a pretested, closed-ended biosecurity questionnaire was taken from the UGBioCheck broiler toolkit (https://biocheckgent.com/en/questionnaires/broilers). A broiler biosecurity toolkit which developed by Ghent university, Belgium was used to quantify the biosecurity measure of the farms. The questionnaires covered both external and internal biosecurity categories annex (VI). External biosecurity (51 questions) comprising of subcategories; purchasing of day-old chicks, feed and water, depopulating of broilers, removing manure and carcass, visitors and farmworkers, material supply, infrastructure and biological vectors and location of farm, and internal biosecurity (28 questions) comprising of subcategories: disease management, cleaning and disinfection, and material and measures between compartments.

The online questionnaire was translated into local language, Tigrigna language, and reproduced for the convenience of both interviewers and interviewees. The translated questionnaire was pretested, and data enumerators were trained online by the Ghent university professionals on how to administer it. Farm owners were invited to participate in a biosecurity questionnaire, and verbal consent was obtained for their involvement. The questionnaire survey was conducted through face-to-face interviews with the farm manager, owner, or attendants. Additionally, the researcher conducted personal observations of the farms beyond using the Tigrigna-translated Biocheck broiler toolkit. The collected hard copy data were entered online at https://biocheckgent.com/en to generate the quantitative scores of the biosecurity practices of each broiler poultry farm.

Biosecurity practices that were thought to have a comparable impact on the possible risk of infectious disease introduction on the farm were merged into a single variable.

The biosecurity score system weighs the various preventive measures in accordance with the efficiency of disease transmission. A balanced perspective on the significance of particular biosecurity measures is provided by the ranking and balancing of the different measures. There are various alternative solutions for every question pertaining to a biosecurity measure, and each response can earn a score between 0 and 10. After combining all of the data, the averages were computed and converted into question and subcategory weights. The system is risk-based, calculating averages and weights at subcategories and individual questions, providing a balanced view of individual measures' importance as previously mentioned by Gelaude *et al*., (2014). Therefore, a final weighted and risk-based score was derived from the biocheck online result for each farm (n=38) based on the various weights assigned to each biosecurity measure and sub-category. This score was calculated by assigning a unique score to each response to a particular question, ranging from 0 (complete lack of preventive measures or full presence of risk) to 1 (complete presence of preventive measures or utter absence of risk). The sub-category result is multiplied by its weight to obtain the subcategory score, as presented in annex (VII).

Farms were classified as "Good/implemented" if the mean score of any component was ≥ 5 and "poor/not implemented" if the scores were < 5 after uniform scaling of each component, and result entered to excel. Then relationships between the disease risk score (coccidiosis) and biosecurity score on each farm for subcategories type were examined using Pearson's correlation coefficient (Greening *et al.*, 2020).

3.5. Data analysis

The raw data collected via the Biocheck survey toolkit from a hard copy questionnaire was processed to yield scores for biosecurity measures: subcategory biosecurity, external biosecurity subtotal, internal biosecurity subtotal, and overall biosecurity for each farm. The scores and coccidiosis infection data were systematically coded and enter into Microsoft Excel spreadsheet for analysis. Descriptive analyses were made to quantify the overall and sub-category biosecurity scores as well as prevalence of coccidiosis. Associations were examined through cross-tabulation, chi-square (χ^2) tests, simple t-test

and logistic regression in STATA version 12, with a significance level set at $P < 0.05$ and a 95% confidence interval.

3.6. Ethical Clearance

Ethical clearance for the study was given by the Animal Experimentation and Ethical review Committee (AEEC) of Mekelle University in a letter dated on November 25,2024 and reference number AEEC 32/024.. The significance of this research was evaluated from ethical perspectives, applicability, and originality technical competence point of view. Moreover, a formal letter was written to poultry farms to get permission and cooperation to conduct the study. Poultry farm owners were requested to participate in the study, and individual informed verbal consent was obtained from poultry in contact human subjects willing to participate in the study. The study participants were informed that participation is solely based on their willingness and any information obtained from participants during the study was kept confidential.

CHAPTER IV: RESULTS

4.1. Farm Characteristics

Since many of these farms were unregistered and did not have formal veterinary supervision, they were able to function without being subject to regulatory inspection. Such a production model delays the early reporting of possible outbreaks and makes it more difficult to build efficient disease control strategies. Every broiler chicken farm in the current study was located 500 meters or less from a residential residence. Furthermore, majority (78.95%) of these farms were located with other poultry farms within the same area. However, only 5.26% of the farms were positioned more than 1000 meters from other neighboring poultry farms. Twenty-three (60.53%) of the chicken farms were small-scale, from which 54.86% of the samples (141/257) collected. On the other hand, 116 samples (45.14%) were from 15 farms (39.47%) that were medium scale. From the total, majority (89.47%) of the farms had concrete floors, and the other farms had earth floors.

4.2. Prevalence of coccidiosis infection

As presented in Table 2, of the 257 examined chicken dropping samples, 23.74% (61) were positive for Eimeria oocysts. Likewise, 68.42% of the assessed farms were tested positive for Eimeria oocysts. Moreover, the result show that farm-level prevalence of coccidiosis is higher than the chicken level prevalence.

Table 2. Overall prevalence of coccidiosis

Characteristics samples		**Result**		**Percentage**
Chicken-level prevalence	257 samples	Positive	61	23.74
		Negative	196	76.26
		Total	**257**	**100.00**
Farm-level prevalence	38 farms	Positive	26	68.42
		Negative	12	31.58
		Total	**38**	**100.00**

4.2.1. Correlation of Coccidiosis with Associated Factors

Of the all assessed predictor variables, the prevalence of coccidiosis infection had a statistically significant associated with the age category of the chickens at 95%CI (4.81 (2.515-9.214) and p= 0.0001) (Table 3**)**.

Table 3. Prevalence of coccidiosis and associated factors

Variables	**Category**	**Total**	**Positive N (%)**	**AOR (95% CI)**	**p-value**
Age	≤8weeks	115	43(16.73)	4.81(2.515-9.214)	0.001
	>8weeks	142	18(7.01)		
Sex	Male	153	38(14.79)	1.24(0.653-2.381)	0.502
	Female	104	23(8.95)		
Floor type	Concrete	244	53(20.62)		
	Soil	13	8(3.12)	2.78(0.815-9.542)	0.059
Farm size	Small	141	36(14.01)	1.15(0.609-2.204)	0.652
	Medium	116	25(9.73)		
Total		**257**	**61(23.74)**		

4.2.2. Frequency of Oocyst per Gram Count (OPG)

As depicted in Table 4, most of the OPG count (70.49%) showed that majority of eimeria oocyte positive chicken had low level of oocyst infection, and only about 10% of the chickens had severe oocyte infection loads.

Table 4. Level of infection based on OPG count

OPG count	Level of infection	No of positive chickens	Percentage (%)
<10000 Oocyst	Low	43	70.49
10000-15000 Oocyst	Medium	12	19.67
>15000 Oocyst	High	6	9.84
Total		61	23.74

4.3. Postmortem Examination

Of the 18 cases examined (12 deceased and 6 executed), 55.56% exhibited gross lesions indicative of coccidiosis gross pathology and tested microscopically positive for oocysts, while 2 had no lesions but were microscopically oocyst positive samples, and 6 showed neither lesions nor microscopic positivity as illustrated in Table 5.

Table 5. Frequency of gross lesion examination

No of chicks examined	Parasitological examination N (%)		Gross lesion Examination N (%)	
18	Positive	Negative	Positive	Negative
	12 (66.67)	6(33.33)	10(55.56)	8(44.44)

N= Number

As shown in table 6, the morphological description of the gross lesions indicated that cecum followed by duodenum was the most affected tissue where high prevalence of cocccidiosis indicative lesions was report (38.89%) and characteristic lesions of hemorrhagic and engorged clotted blood in cecum was presented in Fig.3.

Table 6. Coccidiosis-indicative Gross lesion findings

Site affected	**Nature of gross lesion**	**Number of cases with lesion N (%)**
Duodenum	Hemorrhagic-looking mucosa and white lesions were visible	2 (11.11)
Mid and lower intestine	Petechiae, swelled and inflated with crimson pinpoints	1 (5.56)
Caecum	hemorrhages and engorged clotted blood	7 (38.89)

Figure 3. Hemorrhages and engorged clotted cecum

4.4. Farm Biosecurity Scores

4.4.1. Total Sub-category Biosecurity scores

The average work experience in poultry keeping, number of farm workers, and age of the oldest poultry house in broiler production were 4.11±2.10 years, 1.71±2.10 workers, and 4.79±2.22 years, respectively. The biosecurity scores reported in this study were 44.57% for external measures and 53% for internal measures, resulting in an overall score of 47.26%. Although the overall biosecurity average score (47.26%) and all of its sub-categories fell short of the global average (72%), the disease management sub-category score was relatively high at 70%, while the removal of manure and carcasses scored the lowest at 8.76% compared to the other subcategories (Table 7**)**.

Table 7. Comparison of biosecurity scores of studied farms in relation to the global average

Subcategory	Study average	Global average	P-value
Purchase of day-old chicks	55.68	68	0.001
Depopulation of broiler	37.86	65	<0.001
Feed and water	40.31	63	<0.001
Removal of manure and carcasses	8.76	68	<0.001
Visitors and farm workers	56.10	76	<0.001
Material supply	54.21	70	<0.001
Infrastructure and vectors	50.63	82	<0.001
Location of farm	45.73	68	<0.001
Subtotal external biosecurity	44.57	71	<0.001
Disease management	70.63	80	<0.001
Cleaning and disinfection	38.34	71	<0.001
Materials and measures between compartments	55.6	75	0.001
Subtotal internal biosecurity	53	75	<0.001
Overall biosecurity	47.26	72	<0.001

4.4.2. Routines of External Biosecurity Measure Category

As demonstrated on Table 8, majority of the farms (73.68%) had other animals within the farm compound, while 42.11% of the farm reported there was running or stagnant water within a one-kilometer radius of their farm, and 78.95% of the poultry farms were located within 500 meters of neighboring poultry farms. Furthermore, a significant majority of farms (71.05%) were completely fenced, while only 23.68% reported that they always consider vermin as a problem, with 55.26% acknowledging it as a concern sometimes. Notably, none of the farms reported having workers who also concurrently work on other poultry farms and only 2.63% farm reported that they share materials with other farms. Only 34.21 % of farm reported that their workers wear farm-specific clothing before entering poultry houses, in contrast 81.58 % of workers use only farm-specific footwear. No farms had a separate storage house for carcasses. Only 15.79% of farms had a clear division between clean and dirty areas.

Table 8. The frequency and percentage of routines of external biosecurity

Biosecurity indicators	Category	Number of farms	percentage
Purchase of day-old chicks	Always same supplier	34	89.34
	Sometimes different	4	10.53
day-old chicks first delivered at your farm	Always	26	68.42
	Some times	12	31.58
	Never	-	-
farm site divided into a clean and dirty area	Yes	6	15.79
	No	17	44.74
	I don't know	15	39.47
feed silos sealed against water, birds and vermin	Yes	36	94.74
	No	2	5.26
separate carcass storage house	Yes	-	-
	No	38	100
washing hands after handling carcasses and waste materials	Always	6	15.79
	Sometimes	27	71.05
	Never	5	13.16
Farm worker wear farm specific cloth before entry poultry house	Yes	13	34.21
	No	25	65.79
Farm worker have farm specific shoe before entry poultry house	Yes	31	81.58
	No	7	18.42
farm workers who also work on the other poultry farms	Yes	-	
	No	38	100
material shared with other farms	Yes	1	2.63
	No	37	97.37
Farm fenced	Yes, completely fenced	27	71.05
	Partially fenced	8	21.05
	No	3	7.89
Vermin considered problem on the farm	Always	9	23.68
	Sometimes	1	55.26
	Never	8	21.05
other farm animals being kept on the same farm site	Yes	28	73.68
	No	10	26.32
stagnant or running water within a 1-kilometre radius	Yes	16	42.11
	No	22	57.89
distance nearest neighboring poultry farm	<500 m	30	78.95
	500-1000m	6	15.79
	>1000m	2	5.26

4.4.3. Routines of Internal Biosecurity Measure Category

All farms had a vaccination protocol in place and remove dead birds daily. A high proportion of the farms (97.37%) reported that they clean poultry houses after each production cycle, and 94.74% of them said that they disinfect their farm. Farms implemented a sanitary break after production cycle, with 50% lasting three to eight days and the other half greater than eight days. Almost all (97.37%) of the farms reported that they utilize disinfection as footbaths at farm entrances, and with immediate changes of contaminated fluid (Table 9**)**.

Table 9. The frequency and percentage of routines of internal biosecurity

Biosecurity indicators	**Category**	**Number of farms**	**percentage**
Vaccination protocol	Yes	38	100
	No		
Removal of dead birds from the poultry house	Daily	38	100
	Every two days		
	Less frequent	-	-
Presence of different age categories	Yes	11	28.95
	No	27	71.05
Poultry houses cleaned after each production cycle	Yes	37	97.37
	No	1	2.63
Poultry houses disinfected after each production cycle	Yes	36	94.74
	No	2	5.26
Sanitary break after each production cycle	<3days	-	-
	3-8 days	19	50
	>8days	19	50
Disinfection bath at the entrance of the farm	Yes	37	97.37
	No	1	2.63
Disinfection fluid immediately changed when contaminated	Yes	37	97.37
	No	1	2.63

4.4.4. Adoption of Farm Biosecurity Measure and Coccidiosis Occurrence

The results of the overall farm biosecurity measure applied to control and prevent coccidiosis shows that 63.16 % of the farms had poor biosecurity score. The correlation between the overall risk of coccidiosis occurrence and the overall score of biosecurity shows that the higher prevalence of coccidiosis (57.89%) was found to be statistically associated ($P<0.05$) the poor biosecurity scores of the farms. Considering the correlation between the prevalence of coccidiosis with sub-category biosecurity scores, statistically higher prevalence of coccidiosis ($P<0.05$) was reported in farms with poor subcategory biosecurity scores too (Table 10).

Table 10. Association of farm biosecurity score and occurrence of coccidiosis

Subcategory biosecurity	**Score**	**Farms examined N (%)**	**Positive N (%)**	**X^2**	**p-value**
Farm External biosecurity					
Purchase of day-old chicks	Poor	37(97.37)	26(68.42)	2.225	0.136
	Good	1(2.63)			
Depopulation of broiler	Poor				
	Good	38(100)	26(68.42)		
Feed and water	Poor	37(97.37)	26(68.42)	2.225	0.136
	Good	1(2.63)			
Removal of manure and carcasses	Poor	38(100)	26(68.42)		
	Good	-	-		
Visitors and farmworkers	Poor	14(36.84)	13(34.21)	6.126	0.013
	Good	24(63.16)	13(34.21)		

Table 10…continued

Subcategory biosecurity	Score	Farms examined N (%)	Positive N (%)	X^2	p-value
Material supply	Poor	38(38)	26(68.42)		
	Good	0	0		
Infrastructure and biological vectors	Poor	13(34.21)	13(34.21)	9.120	0.003
	Good	25(65.79)	13(34.21)		
Location of farm	Poor	38(100)	26(68.42)		
	Good	0	0		
Farm Internal biosecurity					
Disease management	Poor	22(57.89)	21(55.26)	17.673	<0.001
	Good	16(42.11)	5(13.16)		
Cleaning and disinfection	Poor	8(21.05)	8(21.05)	4.677	0.031
	Good	30(78.95)	18(47.37)		
Materials and measures between compartments	Poor	38(100)	26(68.42)		
	Good				
Total farm biosecurity score	Poor	24(63.16)	22(57.89)	16.292	<0.001
	Good	14(36.84)	4(10.53)		

CHAPTER V: DISCUSSION

To our knowledge, this study was the first in Ethiopia to measure biosecurity scores and the occurrence of coccidiosis on broiler chicken farms. The sample level and farm level prevalence of coccidiosis in Mekelle were 23.76% and 68.42%, respectively. Majority of the farms were operated with good practices of daily cleaning of feeder and drinker, daily removal of carcass, sanitary break interval between production cycles, good practice in steps of depopulation and no material sharing between farms. These measures are all intended to lower the frequency and severity of infection (Fatoba & Adeleke, 2018a; Yeboah *et al*., 2019; Rony *et al*., 2021). Therefore, a management failure brought on by inadequate knowledge may have prevented the reduction of farm-level coccidiosis prevalence. The sample level prevalence of coccidiosis is closely aligns with findings from Alemayehu *et al*. (2012) in Addis Ababa, which reported a rate of 23.1%, and Yousaf, (2018) in Pakistan, with a prevalence of 23.8%. Additionally, it is comparable to results from Abera *et al.* (2016) at 27.6%, Oljira *et al*. (2012) at 20.57%, Ketema & Fasil, (2019) at 19.5%, Cheru *et al.* (2023) at 26.6% and Ame, (2023) at 27.1% in Addis Ababa, Ambo, Alagae, East Gojjam and Haramaya. However, this finding contrasts with the 65.1% and 42.2% prevalence reported by Gebeyeh & Yizengaw, (2017) and Wondimu *et al*. (2019) from Hawasa and Gondar respectively. The high farm level prevalence of this study was comparatively agreed with a report by from Bangladesh, all (100%) farms were positive to coccidiosis. This study's high farm-level prevalence was comparatively in line with a report from Bangladesh by Rony *et al*. (2021), which found that 100% of farms had coccidiosis. The frequency of coccidiosis, however, ranged from 10 to 90% worldwide Venkatas & Adeleke, (2019), mostly because of variations in location, climate, on-farm cleanliness and biosecurity, production type, and host parameters (species, strains, age, gender, and immunity) (Wondimu *et al*., 2019).

According to the study, the prevalence of coccidiosis declined with chicken age, peaking in those younger than 8 weeks. The prevalence of coccidian oocyst shedding by age group was significantly correlated ($p<0.05$). This is in line to this findings of Ame (2023) from Haramaya, Wondimu *et al*. (2019) from Gondar and Yousaf, (2018) from Pakistan, who reported highest coccidiosis prevalence, i.e. 31.8%, 51% and 31.67% in young age

chickens respectively. The higher prevalence in young birds may be due to their immature immune systems, making them more susceptible to infection by even less pathogenic Eimeria strains (Ola-Fadunsin, 2017).

The study found a significant association between floor type and coccidiosis, with soil type floors having a threefold increase in the risk of developing the disease (p =0.003). However, after adjusting for confounders (other factors) like age, farm size, and sex the association was less pronounced (p=0.102). Soil floors hold moisture and organic matter fostering an ideal environment for coccidia and increasing infection rates Zhou *et al*. (2020), broiler performance, housing disinfection, and thermal discomfort are all negatively impacted by soil floors (Abreu *et al*., 2011). The current study found that 70.49% of hens had a low infection burden, while 19.67% medium and 9.84% high infection levels. In contrast, Wondimu *et al*. (2019) reported a higher percentage of 96.7% low OPG in East Harargae. This discrepancy in findings may be attributed to various factors, including differences in environmental conditions, management practices, and the specific populations of hens studied.

In the present research postmortem examination revealed hemorrhagic mucosa, white lesions in duodenum, petechiae, swollen and inflated mid and lower intestine; hemorrhages, and engorged ceca with clotted blood as illustrated in table 6. In support of current study, Melkamu (2018) also reported similar lessions in *Eimeria tenella*-infected hens in Gondar. Similarly, Balestrin *et al*. (2022) from Brazil described massively thickened cecal walls packed with clotted blood, many petechiae on the serosal surface of the jejunum, and coalescent lesions in the duodenum. Not all of the chicks in the current study tested positive had a microscope developed visible lesions. The reason behind this could be that the degree of Eimeria species infection cannot be reliably determined by macroscopic inspection alone (Santiani *et al*., 2023). The average work experience in poultry keeping, number of farm workers, and age of the oldest poultry house in broiler production were 4.11±2.10 years, 1.71±2.10 workers, and 4.79±2.22 years, respectively. Unlike wise to this result a report by Tanquilut *et al*.(2020), from Philippines 9.6± 9.3 of age of farms and 19.40±11.43 age of farm workers from European countries(Van Limbergen *et al*., 2018). This suggests that broiler production in the research area is relatively young, which contribute to the low biosecurity score. The biosecurity score was

favorably correlated with farm attributes including biosecurity measurements and farm work experience (Tsegaye *et al*., 2023).

The biosecurity average scores for all subcategories of external and internal biosecurity, as well as the overall biosecurity measures, are significantly lower than the global averages. This result is in agreement with Waktole *et al*. (2023), who reported the average overall biosecurity score in the central Ethiopia was lower than the global average, standing at 43.1% compared to 64.3%. However, the current results disagree with reports of Tanquilut *et al.* (2020) who the mean regional biosecurity scores 71.2% compared to 64% in the Philippines which higher than even the global average. Furthermore, the average external and internal biosecurity scores of the farms were lower than those for internal biosecurity, which aligns with findings from previous studies Waktole *et al*. (2023), Elhassan *et al*. (2024), Tanquilut *et al*. (2020, Van Limbergen *et al*. (2018 and Gelaude *et al.* (2014) who reported 40.7%, 52.6% in central Ethiopia, 51%, 75% in Sudan, 68.5%, 77.2% in Philippines, and 68.4%, 76.6% in European countries, respectively. This difference may be due to the positive impact of effective internal biosecurity procedures on performance. Similarly Tablante *et al*. (2002) found that more frequent sanitization of drinking lines improved flock performance, while Van Limbergen *et al*. (2018) noted that better internal biosecurity encourages broiler farmers to maintain higher hygiene standards in broiler houses. Notably, the lowest mean scores of this finding were observed in manure and carcass removal, which recorded a score of 8.66% for external biosecurity, and in cleaning and disinfection, which scored 40.66% for internal biosecurity.

This study revealed that the visitors and farmworkers subcategory in external biosecurity and disease management subcategory in internal biosecurity received the highest score. This is in support of a study finding by Tanquilut *et al*. (2020) from Philippines report that entrance of visitors and farmworkers in external biosecurity had score of 69%, and disease management subcategory in internal biosecurity had the highest score 65.8% in European countries (Van Limbergen *et al.*, 2018). It is advised to implement certain biosecurity precautions as soon as people enter a farm since they can act as a mechanical vector for the spread of infectious diseases (Vangroenweghe *et al*., 2009).

The current study presented that a higher proportion of poultry farmers 73.68% reported washing their hands sometimes after handling carcasses and waste materials, while 13.16% indicated that they always wash their hands. This disagrees with the findings of Waktole *et al*. (2023), which showed that in Addis Ababa, only 50% of farmers washed their hands sometimes and 48.2% always did so after waste disposal. While the study by Waktole *et al*. (2023) in Addis Ababa found that 10.7% of the day-old chicks were delivered from the same supplier consistently and 46.4% occasionally from other sources, this finding shows that more than 3/4th delivery of the purchased chicks came always from the same source and sometimes from a different source 21.05% of the time. Current result obtained about source of chicks shows good, but this may be due to the unavailability different breeder poultry farms. The risk of spreading infections into a flock is increased when chicks are sourced from different farms. Because each source may have varying health conditions, combining them can result in disease outbreaks (USDA, 2019).

The assessment revealed that all farms have established vaccination protocols and consistently remove dead birds daily, aligning with Tadesse *et al*. (2024), which reported (100%) of vaccination practice and 84.31% proper disposal of dead birds across poultry farms in central Ethiopia. The study found that most farms implement promising practices, with having fences, using footbaths at gate entrances, and cleaning poultry houses after each production cycle. While this is lower than the 86% of farms with fences reported in Tsegaye *et al*. (2023), it aligns with the 90.91% of farms using footbaths in the Bishoftu, Ethiopia Ismael *et al*. (2021) and 100% Washing poultry house prior to restocking in Cameroon (Tsabang *et al.*, 2017). Also a previous study in Mekelle by Haftom *et al*. (2015) supports this survey, reporting that 96%, 80%, and 88% of farms practiced chicken vaccination, foot baths at entrances, and cleaning and disinfection of poultry houses, respectively. Combining vaccination with cleaning protocols, including foot baths, enhances biosecurity strategy in poultry farms, minimizing disease introduction risk and improving overall health management (Harrison *et al*., 2022).

Nearly all farms do not permit material sharing among poultry farms. Additionally, more than half of farm workers did not wear special clothing, while 81.58% of farms provided

special footwear for operations. However, a survey by Waktole *et al*. (2023) found that 44.6%, 97.1%, and 94% of the farms in Addis Ababa, Bishoftu, and West of Shaggar, respectively, engaged in the practice of exchanging materials with other farms. To prevent pathogen transfer between and among farms, it's advisable to use farm-specific materials and make them available to anyone in need at the farm (Gelaude *et al*., 2014).

The survey disclosed that more than half of the farms have other animals on-site, frequent animal transport is common, nearly half have water sources within a kilometer, and approximately eighty percent are located within 500 meters of neighboring poultry farms, significantly compromising biosecurity practices. In support of this survey, reports by Waktole *et al*. (2023) and Ismael *et al*. (2021) in Addis Ababa and Bishoftu found that 66.1% and 88.86% of farms were located less than 500 meters from each other and a report from Philippines by Tanquilut *et al*. (2020), 63% of broiler farms had a creek or running water within 1 km. These provide a risk of disease transmission through the air from animals being transported between poultry farms and along public roads. Therefore, the distance to the closest chicken farm should be at least 500 meters, and ideally more than 1 kilometer, should avoid proximity to water bodies where wild waterfowl gather, and distanced from heavily trafficked roads in order to reduce disease transmission (Gelaude *et al*., 2014; USDA, 2019).

The overall farm biosecurity score in this study aligns with findings by Tadesse *et al.* (2024) and Ismael *et al*. (2021), which reported 23.53% and 25% of farms with good biosecurity, and 76.47% and 75% with poor biosecurity in Central Ethiopia and Bishoftu town respectively. However, it disagree with Tsegaye *et al*. (2023), who found 59.3% good and 40.7% poor biosecurity scores in the Arsi and East Showa zones of Oromia, Ethiopia. This poor farm biosecurity score maybe due to lack of economy to invest in biosecurity, inadequate location of farms and lack of training and awareness on the principles of biosecurity measures. It was discovered that biosecurity in commercial farms was impacted by the location, size of the farm flock, biosecurity training, and poultry production experience of farm owners (Tsegaye *et al*., 2023), and the majority of small commercial producers struggle financially (Negro-Calduch *et al*., 2013).

Internal biosecurity components (disease management and cleaning and disinfection subcategories) and external biosecurity components (visitors and farmworkers, infrastructure, and biological vectors subcategories) have a significant correlation with the incidence of coccidiosis ($p<0.05$), and the majority of farms with low biosecurity score subcategories had more coccidian oocysts than farms with higher score biosecurity measures. To support this, a study conducted in Serbia by Pajić *et al*., (2023) found that regular cleaning, disinfection, and all necessary biosecurity measures have a significant ($p < 0.05$) impact on lowering the incidence of coccidia infection and the occurrence of coccidia oocysts in feces samples.According to the research done by Greening *et al*. (2020), one of the most common contact risk pathways for the introduction of diseases in all of the farms they looked into was personnel movement. Likewise, a significant correlation ($p <0.05$) was seen between the presence of eimeria oocysts in this investigation with the visitors and farmworkers subcategory. Only essential personnel enter poultry houses, wearing protective clothing, following a specific order of movement, and keeping detailed records to trace potential infection sources (Allen, 2013).

The present farm level survey indicates that all broiler farms exhibit poor biosecurity score in various subcategories, including farm location, material supply, manure and carcass removal, and materials and measures between compartments. This finding aligns with Elhassan *et al*. (2024), from Sudan who also reports that all broiler farms demonstrate inadequate material supply, with over 80% showing poor practices in manure and dead birds removal, and visitor and personnel access. But this contradicts with report by Greening *et al.* (2020) from New Zealand, that most farms did not share equipment, and of those that did, many reported cleaning it after use. This difference may be due to the high economical and biosecurity principle awareness difference of the developed and undeveloped countries, like our country. Poultry producers can greatly reduce the risk of disease by concentrating on isolation, cleaning procedures, appropriate manure and dead bird disposal, and careful analysis of material supply sources (Trampel *et al*., 2013; Waktole *et al*., 2023).

CHAPTER VI: CONCLUSION AND RECOMMENDATIONS

The findings show that coccidiosis is a significant issue among broiler poultry, affecting many of both the chickens and the farms. Most of biosecurity sub categories were not well adopted in the assessed farms irrespective the farm size. The subcategories of manure and carcass removal, depopulation, and cleaning and disinfection practices were significantly violated. The overall biosecurity score was significantly below the global average. Visitors and farmworkers, infrastructure and biological vectors, disease management, and cleaning and disinfection subcategories show a significant correlation with the risk of coccidiosis. In addition farm-level survey indicates that all broiler farms had poor biosecurity implementation across location of farm, material supply, manure and carcass removal, and, materials and measures between compartmental.

Upon the above conclusions the following recommendations forwarded:

- Before starting organization like broiler poultry farm micro-enterprises, the government should develop biosecurity policies and procedures in the field.
- There is a need to conduct regular training for all farm staff on biosecurity measures, emphasizing the need to reduce cross-contamination between different farm areas and flocks.
- The poor farm level biosecurity score highlights the necessity of increasing biosecurity practice to guarantee the farm's prevention of coccidiosis and other poultry diseases.
- Coccidia isolates should be further characterized by genetic markers.
- Locally feasible options for the control and prevention of Coccidiosis should be in place.

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ANNEXES

Annex I: Fecal sample collection and Record sheet

No	Sub city	Farm code	Farm size	Sample code	Age	sex	Floor type	Result	OPG count

Figure 1: A&B poultry farm with broiler chicken

Annex II: Flotation Method

Preparation of saturated salt solution:

- ✓ Heat 1 liter of tap water to 50-60°C using a warm water bath or a heating plate
- ✓ Weigh and add 375 gram of salt and continue stirring until no more salt dissolves into the solution and excess salt settles on the bottom of the bottle
- ✓ Allow the solution to stand at least 24 h at room temperature to ensure that the solution is fully saturated.

Procedures of fecal floatation examination:

- Place 2 g faeces into a wide-mouthed plastic disposable cup
- Add 44 ml flotation solution to the jar and mix with faeces thoroughly
- Pour/Filter this faecal suspension through a tea strainer into a new jar
- Empty the contents of the jar into a 15 ml test-tube supported in a rack or stand
- Keep adding contents until a positive meniscus forms over the lip of the test tube
- Carefully place a coverslip on top of the test tube
- Stand for 10–15 min
- Carefully lift off the coverslip from the tube, with the drop of fluid adhered to the bottom of it, and place it on a microscope slide.
- Examine oocyst under a light microscope

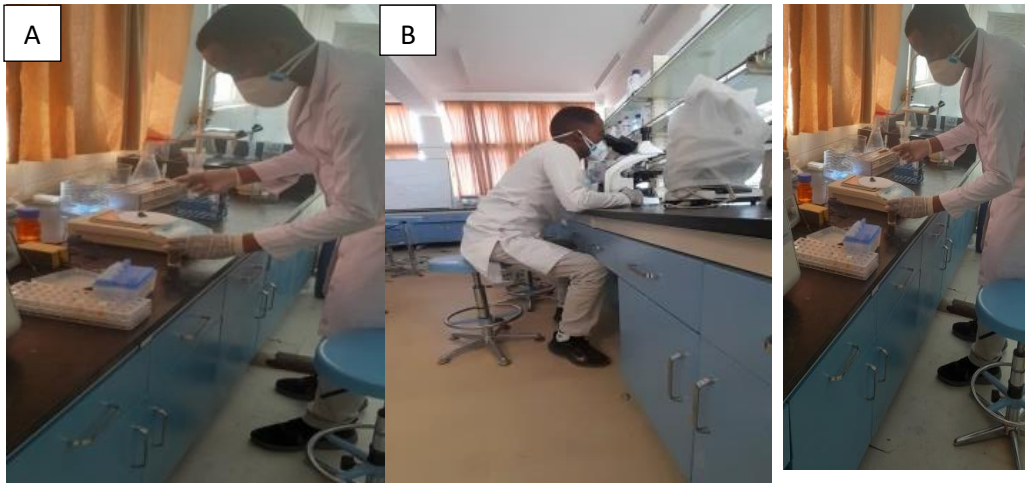

Figure 2: Parasitological examination of chicken droppings

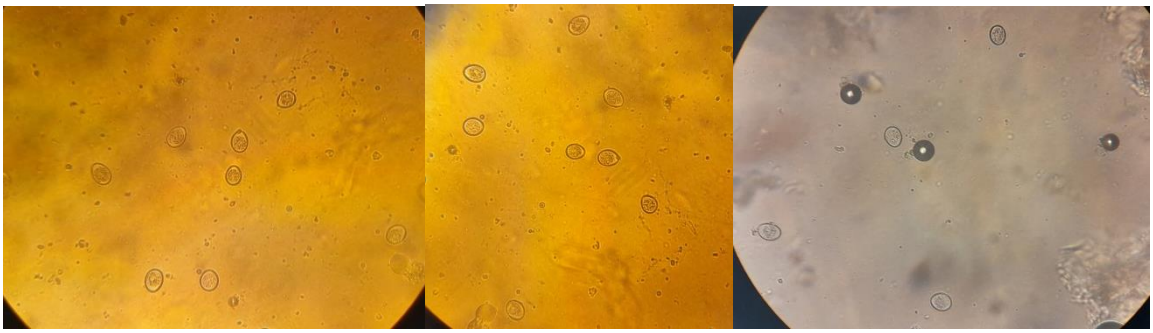

Figure 3: Microscopic Eimeria oocyst

Annex III: McMaster Technique

Procedures:

- ✓ Weigh 2 grams of feces and place into mortar and pestle
- ✓ Add 45 ml of saturated NaCl solution and mix the contents thoroughly
- ✓ Filter the faecal suspension through a tea strainer into a beaker.
- ✓ Using the pipette withdraw a sub-sample as the filtrate is being stirred
- ✓ Stir fluid and fill first compartment of the McMaster counting chamber with the sub sample.
- ✓ Stir fluid again and fill second chamber with another sub sample.
- ✓ Allow the counting chamber to stand for 5 minutes.
- ✓ Examine the subsample of the filtrate under the compound microscope at 10 x 10 magnification..
- ✓ Count the number of oocysts within the grid of each chamber, ignoring those outside the squares
- ✓ Multiply the total by 50 x this gives the oocysts per gram of faeces (o.p.g.)

Annex IV: Cervical Dislocation

Procedures

- ✓ Pick up the bird and hold it until it is calm
- ✓ Place the non-dominant (left hand) over the bird's wings and rest it against hip.
- ✓ By using dominant hand (right hand), grasp the head between thumb and index finger, immediately behind the skull at the mandibles
- ✓ In one swift motion, pull down and twist the head dorsally to stretch the neck and separate the skull from the spinal column.
- ✓ Hold the bird until involuntary movements stop

Annex V: Postmortem examination

Procedures

- Chick humanly kill by cervical dislocation
- The carcass placed in post mortem tray on its back
- Examine for any external injury
- The dead bird soaked in water and place on tray or pm table again
- A cut is made through the corner of the beak with the blunt point of scissors
- The legs stretched outward the way that the legs lie flat on the table.
- The skin from the vent to the neck is cut exposing the muscles.
- From the keel bone the muscles cut from both sides lifting the sternum
- All the visceral organs are exposed
- Internal organs checked properly in situ before removing one by one.
- The GIT is removed carefully by gentle traction and with the help
- The intestines are observed for presence of lesions in the mucosa and in serosa
- Intestinal scraping and cecal scrapings are collected in slides for checking the Eimeria oocyst under microscope.

Figure 4: symptoms of coccidiosis in poultry and poultry house

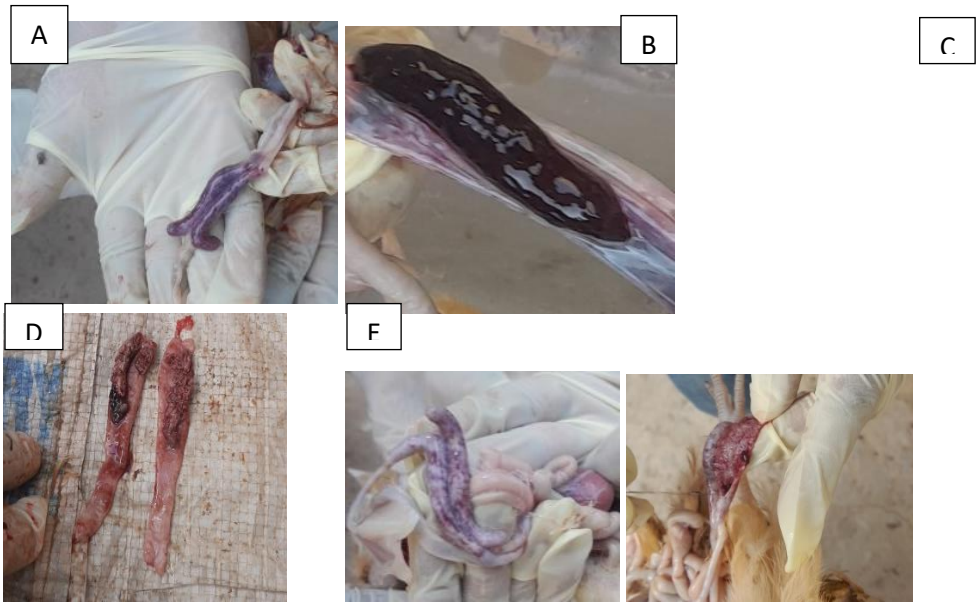

Figure 5: cecum engorged, enlarged and distended (A&D), clotted blood (B), necrotic enteritis (C), hemorrhagic enteritis (E)

Annex VI: Broiler Biosecurity Questionnaire

Farm characteristics

I. How many years of experience in keeping poultry do the person in charge have?

..... Years

II. How many people are working on the farm?

..... Persons

III. How old (in years) is the oldest building in which poultry is being kept?

..... Years

IV. How old (in years) is the newest building in which poultry is being kept?

..... Years

A. Purchase of one-day-old chicks

1. Are your one-day-old chicks (during the last 2 years) always bought from the same original source?

○ Always the same supplier ○ Sometimes a different supplier

2. Are the bought one-day-old chicks first delivered at your farm?

○ Always ○ Sometimes ○ Never

3. Are the transport vehicles cleaned and disinfected before the one-day-old chicks are loaded?

○ Yes ○ No

4. How often a year is one-day-old chicks delivered to your farm?

○ Less than 3 times a year ○ between 3 and 6 times a year ○ more than 6 times a year

B. Depopulation of broilers (slaughterhouses, traders, individuals)

5. Is the transport vehicle for poultry empty on arrival at the farm?

○ Always ○ Sometimes ○ Never

6. Is the transport vehicle for poultry always cleaned and disinfected on arrival at the farm?

○ Yes ○ No

7. Do the driver and the catching team receive and wear farm-specific or disposable clothes/footwear during the loading of poultry?

○ Always ○ Sometimes ○ Never

8. Are individuals and traders allowed to enter the poultry houses where direct contact with the poultry is possible?

○ Always ○ Sometimes ○ Never

9. In how many steps does the depopulation of a poultry house take place?

○ In one step ○ In two steps ○ In more than two steps

10. How often a year is broilers moved from the farm?

○ Less than 6 times a year ○ Between 6 and 12 times a year ○ More than 12 times a year

C. Feed and water

11. Is the farm site divided into a clean and dirty area?

○ Yes ○ No (Go to question 14) ○ I don't know (Go to question 14)

12. Is there a clear separation between the clean and the dirty area of the farm premises?

○ Yes ○ No

13. Can the feeding company fill up the silos/deliver feed without entering the clean area?

. ○ Yes ○ only some of them ○ none

14. Does the feed supplier have access to the houses where direct contact with the poultry is possible?

○ Always ○ Sometimes ○ Never

15. Are the feed silos or the feed storage rooms completely sealed against water, birds and vermin?

○ Yes ○ No

16. How often a year does the feeding company fills up the silos or delivers feed?

○ Less than 20 times a year ○ between 20 and 35 times a year ○ more than 35 times a year

17. How often are bacteriological analyses of the drinking water performed?

○ At least once a year ○ every two years

○ Less frequent than every two years ○ Never (Go to question 19)

18. Where are the water samples for the bacteriological analyses taken?

○ At the source ○ At the last drinker

○ At both locations, i.e. at the source and the last drinker

D. Removal of manure and carcasses

19. Is the manure removed and disposed of appropriately through the dirty road?

○ Yes ○ No

20. Is there separate carcass storage?

○ Yes ○ No (Go to question 25)

21. Can the carcasses be collected by the rendering company without entering the farm premises?

○ Yes ○ No

22. Is the carcass storage space protected from vermin, cats and/or dogs?

○ Yes, it's completely protected ○ It's only partially protected ○ No

23. Is this carcass storage space cleaned and disinfected after each use?

○ Always ○ Sometimes ○ Never

24. Is the carcass storage cooled?

○ Yes ○ No

25. Are carcasses manipulated with gloves, or are hands cleaned and disinfected after manipulation of carcasses?

○ Always ○ Sometimes ○ Never

E. Visitors and farmworkers

26. Are visitors obliged to notify you of their presence before entering the poultry houses?

○ Yes ○ No

27. Do all farmworkers (including the farm owner) abide by the access rules?

○ Always ○ Sometimes ○ Never

28. Is a poultry-free period (longer than 12 hours) expected of all visitors before they are allowed to enter the poultry houses?

○ Yes ○ No

29. Do visitors and farmworkers have to wear farm-specific clothing before they are allowed to enter the poultry houses?

○ Yes ○ No

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

○ Yes ○ No

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

○ Yes ○ No

32. How many times per year is access to the poultry houses granted to visitors?

○ Access is never granted ○ Access is granted, but less than 12 times a year ○ Access is granted more than 12 times a year

33. Are there any farmworkers who also keep poultry or any other type of bird at home?

○ Yes ○ No

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

○ Yes ○ No

F. Material supply

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

○ Yes ○ No

36. Are specific measures taken for the introduction of material?

○ Yes ○ No

G. Infrastructure and biological vectors

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

○ Yes ○ No

38. Is manure being stored on the farm?

○ Yes ○ No

39. Can wild birds enter the poultry houses?

○ Yes ○ No

40. Are bird- and vermin-proof grids placed on the air inlets?

○ Yes ○ No

41. Is the farm fenced?

○ Yes, it's completely fenced ○ It's only partially fenced ○ No

42. Is the outside of the farm (around the walls) paved and clean?

○ Yes, it's completely paved and clean ○ It's only partially paved and clean ○ No

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

○ Often ○ Sometimes ○ Never

44. Is a rodent control programme present on the farm (other than cats)?

○ Yes ○ No

45. Do pets have access to the poultry houses (including the hygiene lock)?

○ Yes ○ No

46. Is “backyard”-poultry or any other type of bird being kept on the farm premises?

○ Yes ○ No

47. Are any other farm animals being kept on the same farm site?

○ Yes ○ No

H. Location of the farm

48. Is there stagnant or running water within a 1-kilometre radius (0.6 miles) of the farm?

○ Yes ○ No

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

○ Less than 500 metres (Less than 0.3 miles)

○ Between 500 metres and 1 kilometre (between 0.3 and 0.6 miles)

○ More than 1 kilometre (more than 0.6 miles)

50. Is manure from other poultry farms spread on the neighboring farmlands?

○ Often ○ Sometimes ○ Never

51. Does animal transport frequently occur?

○ Yes ○ No

I. Disease management

52. Is there a protocol for vaccinations? If so, do you always abide by it?

○ Yes ○ No

53. Is there a regular evaluation made of the disease status of the?

○ Yes ○ No

54. How often are the dead birds removed from the poultry house?

○ Daily ○ every two days ○ Less frequent than once every two days

55. What is the stocking density (according to final weight) of the poultry house?

○ ≤ 33 kg/m² ○ 34 kg/m² ○ 35 kg/m² ○ 36 kg/m²

○ 37 kg/m ○ 38 kg/m² ○ 39 kg/m² ○ 40 kg/m²

○ 41 kg/m² ○ 42 kg/m² ○ > 42 kg/m²

56. Are there different age categories of poultry present on your farm?

○ Yes ○ No

J. Cleaning and disinfection

57. Are vehicle disinfection baths or channels available at the entrance of the farm?

○ Yes ○ No (Go to question 59)

58. Are the vehicle disinfection baths/channels always used?

○ Yes ○ No

59. Are the poultry houses cleaned after each production cycle?

○ Yes ○ No

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

○ Yes ○ No

61. Is the efficacy of cleaning and disinfection checked after each production cycle?

○ Always ○ Sometimes ○ Never

62. Is the loading and unloading area cleaned and disinfected after each production cycle?

○ Yes ○ No

63. How long (in days) does the sanitary break after each production cycle last?

○ Less than 3 days ○ between 3 and 8 days ○ More than 8 days

64. Is there a farm hygiene lock available?

○ Yes ○ No (Go to question 67)

65. Is there a strict separation between the clean and the dirty area of the farm hygiene lock?

○ Yes ○ No

66. Is there a changing room with farm-specific clothes and shoes in the farm hygiene lock?

○ Yes ○ No

67. Is there a house hygiene lock present at every poultry house?

○ Yes ○ No (Go to question 71)

68. Is there a strict separation between the clean and the dirty area of the house hygiene lock?

○ Yes ○ No

69. Is there a disinfection bath/boot washer present in the house hygiene lock?

○ Yes ○ No

70. Is it possible to wash and disinfect your hands in the house hygiene lock?

○ Yes ○ No

71. Is there a disinfection bath/boot washer at the entrance of the farm?

○ Yes ○ No (Go to question 73)

72. Is the fluid of the disinfection baths immediately changed when visually contaminated?

○ Yes ○ No

73. Is the drinking water system properly cleaned and disinfected both on the in- and outside after each production cycle?

○ Always ○ Sometimes ○ Never

74. Are the feeding systems properly cleaned and disinfected both on the in- and outside after each production cycle?

○ Always ○ Sometimes ○ Never

75. Are the feed silos cleaned and disinfected on the inside?

○ Yes, after every one or two production cycle(s) ○ sometimes

○ Never

K. Materials and measures between compartments

76. Is there a protocol for the cleaning and disinfection of material after each production cycle and is this protocol always abided by?

○ Yes ○ No

77. Are there multiple poultry houses present on the farm?

○ Yes ○ No (Go to end)

78. Has clearly recognizable, separate material been foreseen for each poultry house?

○ Yes ○ No

79. Are poultry house-specific clothes and boots available?

○ Yes ○ No

Annex VII: Biosecurity Score of Assessed Farms (N=38)

	PDC	DPB	FEW	RMC	VFW	MAS	IBV	LOF	DIM	CDI	MMC	total
1	3.92	5.39	3.92	3.43	5.39	3.43	5.39	3.43	4.9	6.37	3.43	49
2	3.04	5.39	3.04	2.66	4.18	2.66	4.18	2.66	3.8	4.94	2.66	38
3	4	5.39	4	3.5	5.5	3.5	5.5	3.5	5	6.5	3.5	50
4	4.08	5.39	4.08	3.57	5.61	3.57	5.61	3.57	5.1	6.63	3.57	51
5	4.16	5.39	4.16	3.64	5.72	3.64	5.72	3.64	5.2	6.76	3.64	52
6	2.88	5.39	2.88	2.52	3.96	2.52	3.96	2.52	3.6	4.68	2.52	36
7	3.76	5.39	3.76	3.29	5.17	3.29	5.17	3.29	4.7	6.11	3.29	47
8	3.52	5.39	3.52	3.08	4.84	3.08	4.84	3.08	4.4	5.72	3.08	44
9	3.68	5.39	3.68	3.22	5.06	3.22	5.06	3.22	4.6	5.98	3.22	46
10	4.24	5.39	4.24	3.71	5.83	3.71	5.83	3.71	5.3	6.89	3.71	53
11	3.2	5.39	3.2	2.8	4.4	2.8	4.4	2.8	4	5.2	2.8	40
12	3.2	5.39	3.2	2.8	4.4	2.8	4.4	2.8	4	5.2	2.8	40

	PDC	DPB	FEW	RMC	VFW	MAS	IBV	LOF	DIM	CDI	MMC	total
13	4.4	5.39	4.4	3.85	6.05	3.85	6.05	3.85	5.5	7.15	3.85	55
14	4.4	5.39	4.4	3.85	6.05	3.85	6.05	3.85	5.5	7.15	3.85	55
15	4.24	5.39	4.24	3.71	5.83	3.71	5.83	3.71	5.3	6.89	3.71	53
16	4.4	5.39	4.4	3.85	6.05	3.85	6.05	3.85	5.5	7.15	3.85	55
17	5.04	5.39	5.04	4.41	6.93	4.41	6.93	4.41	6.3	8.19	4.41	63
18	3.04	5.39	3.04	2.66	4.18	2.66	4.18	2.66	3.8	4.94	2.66	38
19	2.96	5.39	2.96	2.59	4.07	2.59	4.07	2.59	3.7	4.81	2.59	37
20	4.16	5.39	4.16	3.64	5.72	3.64	5.72	3.64	5.2	6.76	3.64	52
21	3.92	5.39	3.92	3.43	5.39	3.43	5.39	3.43	4.9	6.37	3.43	49
22	2.96	5.39	2.96	2.59	4.07	2.59	4.07	2.59	3.7	4.81	2.59	37
23	2.72	5.39	2.72	2.38	3.74	2.38	3.74	2.38	3.4	4.42	2.38	34
24	4.08	5.39	4.08	3.57	5.61	3.57	5.61	3.57	5.1	6.63	3.57	51
25	4.24	5.39	4.24	3.71	5.83	3.71	5.83	3.71	5.3	6.89	3.71	53
26	3.68	5.39	3.68	3.22	5.06	3.22	5.06	3.22	4.6	5.98	3.22	46
27	4	5.39	4	3.5	5.5	3.5	5.5	3.5	5	6.5	3.5	50
28	2.64	5.39	2.64	2.31	3.63	2.31	3.63	2.31	3.3	4.29	2.31	33
29	3.84	5.39	3.84	3.36	5.28	3.36	5.28	3.36	4.8	6.24	3.36	48
30	2.8	5.39	2.8	2.45	3.85	2.45	3.85	2.45	3.5	4.55	2.45	35
31	4.64	5.39	4.64	4.06	6.38	4.06	6.38	4.06	5.8	7.54	4.06	58
32	3.6	5.39	3.6	3.15	4.95	3.15	4.95	3.15	4.5	5.85	3.15	45
33	3.44	5.39	3.44	3.01	4.73	3.01	4.73	3.01	4.3	5.59	3.01	43
34	4.88	5.39	4.88	4.27	6.71	4.27	6.71	4.27	6.1	7.93	4.27	61
35	4.48	5.39	4.48	3.92	6.16	3.92	6.16	3.92	5.6	7.28	3.92	56
36	3.92	5.39	3.92	3.43	5.39	3.43	5.39	3.43	4.9	6.37	3.43	49
37	3.76	5.39	3.76	3.29	5.17	3.29	5.17	3.29	4.7	6.11	3.29	47
38	3.76	5.39	3.76	3.29	5.17	3.29	5.17	3.29	4.7	6.11	3.29	47

PDC = purchase of day old chick; DPB= depopulation of broilers; FEW = feed and water; RMC= removal of manure and carcass; VFW = visitors and farmworkers; MAS= material supply; IBV=infrastructure and biological vector; LOF=location of farm; DIM=

disease management; CDI= cleaning and disinfection; MMC= material and measures between compartments

Annex VIII: Biosecurity Measures Combined Subcategories

A. External Biosecurity Measures

1. Purchasing of day old chicks (PDC): weight (8)

Refers; source of purchased chicken, delivery of chicken first on farm, transport vehicles cleaned and disinfected before the day-old chicks are loaded and frequency of chicks deliver on farm yearly

2. Depopulation of Broiler (DPB): **weight (11)**

Includes; delivery of transport vehicle empty on farm and, clean and disinfect on arrival; yearly frequency and steps of depopulation chicks; management of individuals and traders direct contact with poultry

3. Feed and Water (FEW): weight (8)

Indicates; is farm divided into clean and dirty with clear separation, sealed feed storage against water and vermin and bacteriological analysis of drinking water

4. Removal of Manure and Carcass (RMC): **weight (7)**

Comprising of; separated carcass storage house, way of handling manure and carcass, habit of use of gloves and hand washing after manure and carcass manipulations

5. Visitors and Farmworkers (VFW): **weight (11)**

Practices consist of; obligation of visitors to notify before entry, farm specific clothes and boots for visitors and farmworkers, hand washing and disinfection during farm entry, employees working in different farms

6. Material Supply (MAS): weight (7)

Encompasses; material sharing practices and disinfect shared materials before and after use

7. Infrastructure and biological vector (IVF): **weight (11)**

States; access of birds to outside (open air), wild bird access to farms, manure store in farm, and access of pet animals (cats and dogs), fence of farm, rodent control program of the farm and other animal on farm site

8. Location of Farm (LOF): weight (7)

Explains; stagnant water presence, the position of farm relative to neighbor poultry farm, relative location from main road, manure of other poultry farm spread on neighbor farmlands

B. Internal Biosecurity Measures

9. Disease Management (DIM): **weight (10)**

Deals with; vaccination protocol, regular evaluation health status, removal of dead birds, stocking density and different age categories present on farm

10. Cleaning and Disinfection (CDI): weight (13)

Represents to; vehicles disinfection baths, poultry house cleaning and disinfecting after production cycle, footbath facility, frequency of changing footbath, sanitary break after production cycle, farm and poultry house hygiene lock and cleaning and disinfection of farm materials.

11. Material and Measures between Compartments (MMC): weight (7)

Refers to; protocols for cleaning and disinfection material after each production cycle, multiple poultry houses and clearly recognizable separate material for each poultry houses

Mekelle University

Animal Ethics and Experimentation Committee (AEEC) Clearance Letter

Date: November 25, 2024

To: Tesfay Gebrewahd, Principal Investigator (PI)

Mekelle

Subject: Notification of AEEC decision on your research proposal

AEEC No: AEEC 32/2024

Protocol (Title of the study): Measuring Biosecurity Practices and Occurrence of Coccidiosis in Broiler Farms in Mekelle City, Tigray

Dear PI,

The aforementioned research proposal which was submitted by you for animal ethics and use clearance letter has been reviewed by the Animal Ethics and Experimentation Committee (AEEC).

The AEEC has discussed and examined the research proposal in detail from animal ethics and use principles and values perspective.

Finally, the AEEC has (**approved**, approved with modification or withhold approval) your research proposal.

This animal ethics clearance letter is valid for only one year (25/11/2024 – 25/11/2025).

Furthermore, any other correspondence and inquiries concerning your research proposal with committee must include the AEEC No., the name of the PI and the proposal title.

Best Regards,

AEEC Chairperson

Name: Nigus Abebe (PhD)

Signature:

Date: 25/11/2024

AEEC Secretary

Name: Enquebaher Kassaye (PhD)

Signature:

Date: 25/11/2024

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