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PREVALENCE AND MAGNITUDE OF INTESTINAL NEMATODES AND ANTHELMINTIC EFFICACY AGAINST THESE PARASITES IN CHICKENS IN BISHOFTU, ETHIOPIA

MSC THESIS

BY

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DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH

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By

Abdulkerim Yusuf

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APPROVAL SHEET

PREVALENCE AND MAGNITUDE OF INTESTINAL NEMATODES AND ANTHELMINTIC EFFICACY AGAINST THESE PARASITES IN CHICKENS IN BISHOFTU, ETHIOPIA

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LIST OF ABBREVATION AND ACRONYMS

AR	Anthelmintic Resistance
BMZ	Benzimidazoles
DNA	Deoxyribonucleic Acid
EPG	Eggs per Gram
FBZ	Fenbendazole
FEC	Fecal Egg Count
FECRT	Fecal Egg Count Reduction Test
GIN	Gastrointestinal Nematodes
LCL	Lower Confidence Limit
MF	Mini Flotac
PIP	Piperazine
UCL	Upper Confidence Limit

ABSTRACT

Poultry production is emerging as a highly developed sector of global food animal production. However intestinal nematode infection is one of significant challenges to the poultry industry due to their health, production and welfare impacts. A cross-sectional study design was used to investigate the prevalence and magnitude of intestinal nematode infections in chickens kept under commercial and backyard production systems in the Bishoftu town from November 2024 to June 2025. For this purpose, layer, dual purpose chicken and broiler flocks were included. During sample collection, data such as poultry types, flock size, breed, and management systems were recorded. In addition, anthelminthic efficacy of piperazine and fenbendazole was evaluated. A pooled fecal sample was collected from 210 chicken farms for fecal egg counts and 115 gastrointestinal tracts of chickens were examined for worm counts. The overall prevalence of Ascarid eggs based on Mini FLOTAC technique was found to be 63.8%. Factors such as poultry types and flock management were found to have a statistically significant ($p < 0.05$) influence on the prevalence of the parasites. Similarly, significant differences ($p<0.05$) were found in the mean EPG across poultry types and breeds of the flocks. The mean worm counts were nearly similar in layer and dual-purpose chicken for *A. galli* and *H. gallinarum* worms, while broilers harbour much lower worm counts. The anthelmintic test results showed that fenbendazole and piperazine are 100% effective at recommended dose via drinking water, indicating that anthelmintic resistance is not concern in the study area. In conclusion, this study strongly suggested that *A. galli* and *H. gallinarum* is highly prevalent, which might impact the productivity of the chickens. Therefore, it is essential to implement effective control measures and maintain regular deworming with effective anthelmintics such as fenbendazole and piperazine in poultry farms, to manage intestinal nematodes based on infection intensity.

Keywords: *Bishoftu; Infection intensity; Intestinal nematodes; Poultry; Prevalence*

1. INTRODUCTION

Poultry production is emerged as highly developed sector of the global food animal production (Jez *et al*., 2011). Today, chicken rearing dominates the production of poultry to a great extent (Midala *et al*., 2025). Poultry production, marketing, and consumption experience lesser social and religious taboos than other livestock species. As a result, chicken and other poultry commodities are a leading global protein source of human diets (Birhanu *et al*., 2023). They are quite important to the life of humans because they ensure food security via meat and egg production, earn people income, and maintain social-cultural roles (Ngongolo *et al*., 2021).

Poultry can be found in backyard settings or commercial production systems across the world (Mottet and Tempio, 2017). Ninety-five percent of chicken production in Ethiopia is carried out through free scavenging production, in contrast to the intensive management systems used in wealthier countries (Belete *et al*., 2016). However, intensive poultry management is currently thriving due to policy shifts towards privatization and mixed economies. In spite of the large number of chickens, poultry sector in the country faces significant challenges due to high prevalence of diseases, poor veterinary services and poor biosecurity (Tolossa and Tafesse, 2013).

Intestinal nematode parasites are one of major concern for the poultry industry worldwide due to their health, welfare and production impacts (Berhe *et al*., 2019; Tsegaye and Miretie, 2021). This is of particular importance in Ethiopia where climatic condition and poor biosecurity standards favours their development (Mekuria and Bayessa, 2017). The most prevalent nematodes are *Ascaridia galli (A. galii), Heterakis gallinarum (H. gallinarum)* and *Capillaria species* (Afolabi *et al*., 2016). *A. galii* infection is the most common parasitic disease affecting chickens, particularly in developing nations, followed by the caecal nematode *H. gallinarum* (Sharma *et al*., 2019; Shifaw *et al*., 2021a; Tusa, 2023).

The disease caused by these parasites has been associated with various health issues and a decline in productivity. This includes disruption of gastrointestinal barrier, decreased feed conversion ratio, reduced weight gains and weight loss in broilers, poor egg lay in layers, and increased mortalities (Sreedevi *et al*., 2016; Ola-Fadunsin *et al*., 2019). In addition, the parasites themselves can also act as a vector for other

infectious agents such as Salmonella and Histomonas, which are transmitted by *A. galli* and *Heterakis* spp., respectively. Worm infections mainly pose a problem in farms with a primary activity in egg production, simply because the birds are kept for a longer period of time (Dube *et al*., 2010). Furthermore, these parasites undermine the immune system or negatively influence the efficiency of administered vaccines which can make the flock less resistant to diseases and exacerbate existing disease conditions. The magnitude of these effects can vary depending on factors such as the infected nematode species, degree of intensity of infection, management practices, and immune status of the flock (Shifaw *et al*., 2021a).

Despite their significant impacts, intestinal worm infections in poultry have received little research attention for many years; so much of the literature involves case reports or superficial surveys. Research and control efforts in Ethiopia have largely focused on bacterial and viral diseases, with little attention to these worms. Some screening studies in different parts of Ethiopia demonstrated a high prevalence of almost 90-100% of mixed infection with helminth parasites in backyard laying hens, with *A. galli* and *H. gallinarum* as dominant species, almost occurring in 70% of the sampled animals (Mebrahtu *et al*., 2019). However, there is no data on the epidemiology of nematode infections in chicken flocks kept under commercial farming systems in the country. The current shift in poultry farming from cage systems to deep litter management has dramatically increased the risk of intestinal worm infections. This system creates favorable conditions for the lifecycle completion and accumulation of gastrointestinal nematode eggs leading to increased infection and burden (Maurer *et al*., 2009).

Effective control of nematode parasites is accomplished through good management strategy, with other control approaches (Zloch *et al*., 2018). However, the scarcities of veterinary services, poor standards of housing and major weaknesses in biosecurity are some of the challenges for the control of these parasites. Currently, anthelmintic drugs are the basis for the management of worm infection and will probably remain so in the future due to the general lack of antiparasitic vaccines. Typical anthelmintic drugs used in the treatment of nematode infections in poultry are piperazine, albendazole, fenbendazole and ivermectin (Feyera *et al*., 2022). However, in recent years, resistance to major classes of broad-spectrum anthelmintics has been reported

worldwide, including in many African countries due to a limited number of anthelmintic products worldwide (Tarbiat *et al*., 2017). Reduced efficacy against parasites of extensively used anthelmintics, including fenbendazole and levamisole has been documented in laying hens and turkeys, in other country (Yazwinski *et al*., 2009, 2013). However, the efficacy of these drugs has never been confirmed in Ethiopia. Hence, a comparative evaluation of the efficacy of the commonly used anthelmintic drugs in Ethiopia, alongside the recommended drugs in other countries (fenbendazole), is necessary under controlled conditions before implementing mass-deworming programs. This approach would ensure the adoption of the most effective and sustainable treatment options for parasite control to enhance poultry health and productivity. Therefore, the objectives of the study were:

General objective

- To estimate the prevalence and intensity of intestinal nematodes and evaluate the therapeutic efficacy of fenbendazole and piperazine drugs against these parasites on naturally infected chickens in study area.

Specific Objectives

- To estimate the prevalence and magnitude of intestinal nematodes among commercial and backyard poultry farms of study area.
- To evaluate and compare the treatment efficacy of fenbendazole and piperazine against the intestinal nematodes based on reduction in fecal egg counts and postmortem worm counts.

2. INTESTINAL NEMATODES OF POULRTY

2.1.Major Species and Their Description

2.1.1. Ascaridia galli

Ascaridia galli is the most common species and pathogenic nematode species, particularly in chickens, and leads to the development of ascaridiosis disease (Shifaw *et al*., 2021a). This parasite infects chickens but may also infect other birds (Jaiswal *et al*., 2020; Zloch *et al*., 2021). It is the large nematode species infecting poultry, particularly young birds (Alemu, 2021). Adult worms measure as long as one and a half to three inches, about the same size as an average pencil lead, so they are easily visible with the naked eye (Tarbiat, 2021). The worms of this nematode are also semi-transparent, with the female 72 to 116 mm and male 51 to 76 mm in length, being the largest nematode in poultry. There are three big lips on the oral opening, tail is straight with a caudal spine and measures 0.65–0.95 mm in length. These worms primarily reside in the small intestine of birds (Belete *et al*., 2016). The Eggs are large, oval and measures 0.055–0.065 mm in length and 0.04–0.05 mm in width (Tanveer *et al*., 2015). The larvae of *A. galii* have a distinctive morphological description. The larvae are generally cylindrical body elongate with pointed head and tapering tail, measuring about 1-2mm in length and 0.1-0.2mm in diameter (Decraemer *et al*., 2023). Note that the morphological description of larvae could be different based on developmental stage as well as environmental conditions (Lalchhandama *et al*., 2010).

2.1.2. Heterakis gallinarum

Heterakis gallinarum is an internal parasite that infects the ceca of birds. It is a small roundworm, and a member of the family *Heterakidae* (Kaufmann *et al*., 2011). Adult worms possess a minute body measuring approximately 7-13 mm in the case of males and approximately 10-15 mm in the case of females. They possess a cylindrical elongated body with tapering at the two ends. The mouth is lined by three well-formed lips, and the esophagus is club-shaped and without a posterior bulb (Cupo and Beckstead, 2019a; Abdullah *et al*., 2021). The eggs, when being expelled from the female body, are oval and measure roughly 65-80 µm x 35-50 µm. They possess a hard, smooth cuticle and a single cell inside (Kerroucha *et al*., 2022). The typical

morphological features of *H. gallinarum* larvae will generally vary slightly depending on host species, location, and parasite strain. They usually have a cylindrical body, tapered tail, and pointed head all covered by a tough, thick exterior coat of cuticle (Bobrek *et al*., 2019).

2.1.3. Capillaria species

Capillariasis is the name of the disease caused by infection with Capillaria species, which are a type of internal parasite (El-Ghany, 2022). There are several different species of Capillaria; each invades a specific region inside the chicken. The worms reside in the small intestine and in the caeca, depending on the species. They are also found in the crop and oesophagus (Kaufmann, 2013). Adult Capillaria are very thin, "hair-like" nematodes, which are approximately 1 cm (0.39 in) long. Their eggs, which can only be seen using a microscope, are barrel-shaped, colourless and have a thick shell that is slight striated with bipolar plugs. Larval Capillaria parasites are typically microscopic and challenging to observe without magnification. They possess a long, slender body that has a thread-like appearance and exhibit limited movement compared to other nematodes (Decraemer *et al*., 2023).

2.2.Epidemiology of Intestinal Nematodes in Chicken

2.2.1. Distribution and prevalence

Nematodes of poultry are distributed all over the world. Geographical distribution varies geographically from region to region depending upon various factors, like climate, and other suitable factors (Trisha *et al.*, 2021). Most of the studies that reported on the prevalence of poultry nematodes were primarily reporting various types of intestinal nematode infections (Mathews *et al*., 2024). The disease continues to be of high significance in traditional system, deep-litter and free-range commercial production systems (Kaufmann *et al*., 2011).

Distribution of *Ascaridia galli*: It is widely prevalent in backyard and commercial layer production systems globally (El-Ghany, 2022). Hens that have been co-infested with *A. galli* and other nematodes in the intestine, along with some other problems, cause enormous issues in chicken production, particularly in free-range conditions (Bettridge, 2014). Several studies have revealed the high prevalence of *A. galli* in

commercial and backyard poultry farms around the globe. Lots of studies have been conducted to report the prevalence and distribution of *A. galii* in Asian nations. Mathews *et al*. (2024) had already reported prevalence rate of (45.33%) in India. Another study conducted by Eslami *et al*. (2009) had already reported prevalence rate of 56% in Iran. 62.3 % of India (Javaregowda *et al*., 2016). Prevalence of ascaris parasite in poultry farm became common among European countries. For instance, 63.8% in Denmark (Permin *et al*., 1999), 88% in Germany (Kaufmann *et al*., 2011), 84% in England and Wales (Sherwin *et al*., 2013) and 97% in Netherlands (Thapa *et al*., 2015).

The challenges of this worm were also reported in multiple reports conducted on backyard chicken from different regions of Africa. For instance, a study reported that 69% of growers' chicken in Tanzania (Magwisha *et al*., 2002), another study reported a percentage rate of 58.3% from South Africa (Mlondo *et al*., 2022) and 33.3% in Kenya (Mungube *et al*., 2008). A study conducted in Ethiopia reported an *A. galli* infection prevalence of 90.21% in village chickens (Ashenafi and Eshetu, 2004) and El-Dakhly *et al*. (2019) reported an infection prevalence of 55.79% in Egypt.

Table 1: Distribution of *Ascaridia galli* reported in chickens

Country	**Production Type**	**Methods**	**Prevalence**	**Reference**
Germany	Extensive	Necropsy	96.2%	Wongrak *et al*., 2014
Palestine	Free range	Necropsy	75.6%	Rayyan *et al*., 2010
India	Extensive	Necropsy	34.25%	Ara *et al*., 2021
Ghana	Both	Floatation	32.5%	Asumang *et al*., 2019
Bangladesh	Extensive	Necropsy	56%	Trisha *et al*., 2021
England	Free range	Floatation	84%	Sherwin *et al*., 2013
Ghana	Extensive	Molecular	37.2%1	Anane *et al*., 2022
Ethiopia	Backyards	Necropsy	69.8%	Sarba *et al*., 2019
Ethiopia	Both	Necropsy	68.84%	Berhe *et al*., 2019

Distributions of *Heterakis gallinarum*: *Heterakis gallinarum* is a worldwide nematode parasite with the preference to infect avian hosts frequently, especially domestic chickens and other birds belonging to family Galliformes (Biswas *et al*.,

2021). Although the exact prevalence varies depending on a number of factors, it has also been known to be prevalent in commercial laying hen production systems (Vijayalingam *et al*., 2020). Studies have investigated on the prevalence of *H. gallinarum* in chickens, reporting prevalence rates ranging from 4% to 98 % (Tarbiat *et al*., 2021). In Europe, a total prevalence of 29.0% of *H. gallinarum* infection is described, with significant differences between countries (Thapa *et al.*, 2015). In a study in Northern Italy, *H. gallinarum* recorded the highest infection rate of 95.7% among nematode parasites (Wuthijaree *et al*., 2017).

Different studies have reported different percentages of prevalence of this infection in African nations. For instance, in Ethiopia, 72.5% prevalence was reported in free-range chickens in a survey (Berhe *et al*., 2019), and in South Africa, 80-94.4 % prevalence was reported in free-range chickens in a study (Mukaratirwa and Khumalo, 2010). In North-western Algeria, 78.07% prevalence was reported in local chicken (Yousfi *et al.*, 2013). In a recent study in Australia, 87% infection rate was reported (Shifaw *et al*., 2023). Prevalence studies conducted in most Asian countries all reported different prevalence of *H. gallinarum* infection in poultry, for example, 38.89% (Khan *et al*., 2016), 28.70% (Abdullah *et al*., 2021), 22.5% (Ara *et al*., 2021), and 7.4% (Jaiswal *et al*., 2020), 59.33 % (Mathews *et al*., 2024).

Table 2: Distribution of *Heterakis gallinarum* reported in chicken

Country	**Production Type**	**Methods**	**Prevalence**	**Reference**
Germany	Organic Free range	Post-mortem	98%	Kaufmann *et al*., 2011
Italy	Free Range	FEC	95.7%	Wuthijaree *et al*., 2017
Australia	Free range layer	Post-mortem	87 %	Shifaw *et al*., 2023
Thailand	Extensive	Molecular	86.7%	Butboonchoo *et al*., 2017
India	Extensive	Necropsy	59.33%	Mathews *et al*., 2024
Colombia	Backyards	MacMaster	59.4%	Montes-Vergara *et al*.,2021
Brazil	Free range	Floatation	100%	Silva *et al*., 2022
Ethiopia	Intensive and backyard	Necropsy	72.5%	Berhe *et al*., 2019
Ethiopia	Commercial	Flotation	57%	Adamu *et al*., 2019
Philippines	Commercial	Floatation	59.3%	Ybañez *et al*., 2018

Distributions of Capillaria: chicken capillariasis, a nematode parasitic group infecting various bird species, including chickens. Prevalence and distribution of

Capillaria species vary widely among various geographical regions and farming systems. In tropical and subtropical parts of the world such as Latin America, Southeast Asia, and Africa, the hot and humid environment is best for the growth cycle of Capillaria, thereby enhancing infection rates (Adang *et al*., 2010). For instance, in Brazil, studies conducted have indicated high infection levels in areas with free-range poultry production systems, where the chicken would be more exposed to infected soil (Oliveira *et al*., 2009). On the other hand, temperate climate regions like Europe and North America typically possess lower prevalence rates that are due to intensive farming practices and improved biosecurity measures. Prevalence rate of 75.3 % Capillaria species was, however, noted in Germany by (Kaufmann *et al*., 2011). A study in Ethiopia showed a 24.6% prevalence rate under free-ranging systems in chickens (Eshetu *et al*., 2001). Conversely, in Nigeria a 65.33% prevalence rate was recorded by (Ngwamah *et al*., 2024).

Table 3: Distribution of *Capillaria Species* reported in chicken

Country	**Production Type**	**Methods**	**Prevalence**	**Reference**
Austria,	Commercial	Floatation Method	39.4 %	Zloch *et al*., 2021
Germany	Organic free range	Necropsy	75.3%	Kaufmann *et al*., 2011
Germany	Extensive	Necropsy	86.1%	Wongrak *et al*., 2014
Australia	Free range layer	Necropsy	34.9%	Shifaw *et al*., 2023
Colombia	Backyards	MacMaster Techniques	45.6%	Montes-Vergara *et al.*, 2021
Italy	Free range	FEC	66.8%	Wuthijaree *et al*., 2017
Mexico	Backyards	McMaster technique	46.5%	Cervantes-Rivera *et al*., 2016
Nigeria	Caged chicken	Floatation	25.33	Ngwamah *et al*., 2024

2.2.2. Life cycle and transmission mechanisms

The life cycle of *A. galli* is simple and direct where the infective eggs (egg contains Second stage larva) hatch in the duodenum of bird (Figure 1) (Höglund *et al*., 2023). The larvae burrow through lining intestinal epithelium and form two moults. Then, they develop into adults and produce large quantities of eggs that excreted in the

droppings of birds and continue their life cycle. The prepatent period of *A. galli* is 5 to 6 weeks (Shohana *et al*., 2023). *H. gallinarum* life cycle is similar to that of *A. galli*, but it exhibit the indirect life cycle and inhabit caecal portions of large intestines. In case of *H. gallinarum*, the development into mature worms after infection takes approximately two weeks. The prepatent period for *H. gallinarum*, which is the time from ingestion of infective eggs to the production of eggs by the adult worms, is around 4-6 weeks (Cork and Lejeune, 2019). Similar to *H. gallinarum*, *Capillaria spp*. have both direct and indirect life cycles, with earthworms required for the completion of their life cycle. The prepatent period for *Capillaria spp*. is approximately three weeks (Belete *et al*., 2016).

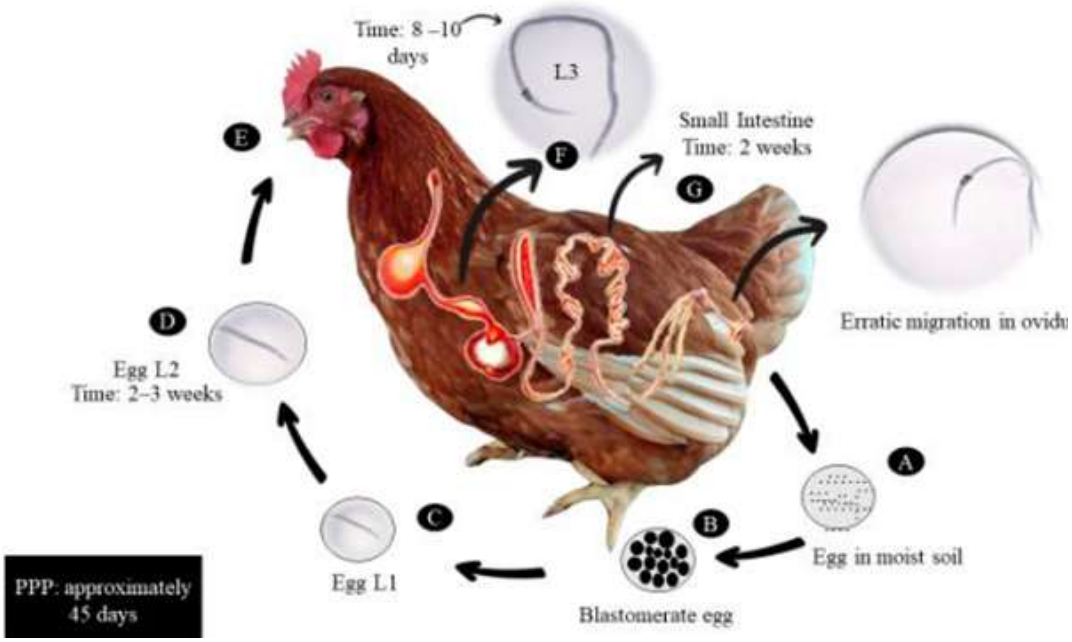

Figure 1: *Ascarids* worm life cycle (Bautista-Vanegas *et al*., 2023).

The primary mode of transmission of the principal gastrointestinal nematode parasite is through fecal-oral route (Vijayalingam *et al*., 2020; Sharma *et al*., 2019). Although, transmission among birds may also occur, as fecal-contaminated chickens can transfer eggs directly to other chickens by contact or pecking at each other's feces. Nevertheless, *H. gallinarum* and some Capillaria species transmit indirectly, which is usually earthworms (figure 2) (McDougald, 2020). Such a transmission mode can enhance the transmission of Heterakis species in poultry production (Abdullah *et al*., 2021).

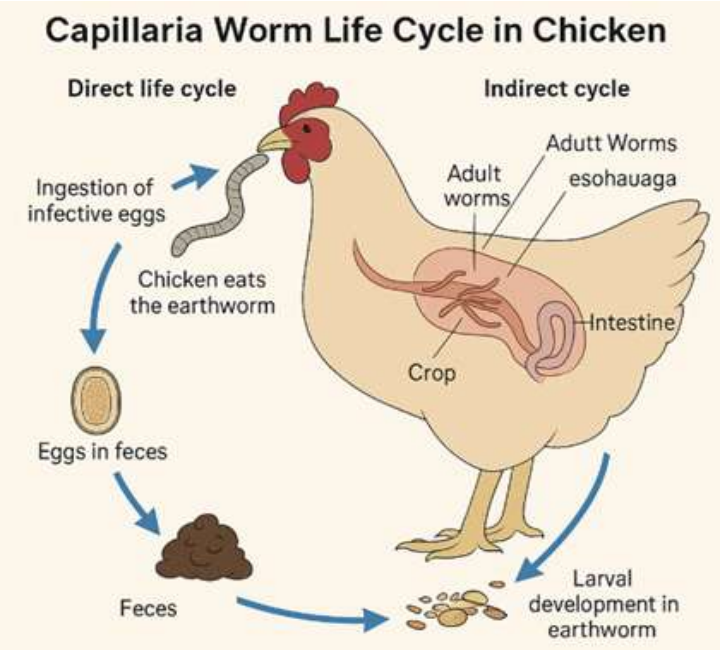

Figure 2: Capillaria worm life cycle (Luke, 2020).

2.2.3. Factors influencing the spread and transmission

The distributions and intensity of parasite infections can be influenced by a number of factors including availability and infection level of their intermediate hosts, and the population density of infective developmental stage (Ebrahimi *et al*., 2014). Host-related factors, such as breed, and age, can also influence the infection. The variation in the worldwide prevalence of gastrointestinal nematodes in chicken flocks is determined by geographical location, climate, and management (Shifaw *et al*., 2023).

Parasite related Factors: Firstly, it is well known that parasite eggs are resistant to harsh environmental conditions (Tarbiat *et al*., 2018). Survival time of eggs of nematodes in the environment varies, but generally, they can survive for several years to months under existing environmental conditions (Morand *et al*., 2006). Unlike fragile eggs of capillaria species, ascarid eggs (Ascarida/Heterakis species) have a resistant shell structure that persists in soil for years (Taylor *et al*., 2007; Moravec, 2009). The eggs of intestinal ascarid parasites (Ascaridia and Heterakis spp.) of chickens can survive long-term in soil and this makes contaminated yards and pastures infective to chickens for years (Thapa *et al*., 2017). It undergoes metabolic dormancy by reducing their metabolic activity, allowing them to conserve energy and survive for extended periods (Kiontke and Fitch, 2013). Earthworms serve as intermediate hosts for *H. gallinarum*, a parasitic infection (Lund and Galtung-Hansen, 2020). Chickens

that consume infected earthworms themselves become infected. This poses a higher risk for free-range flocks and those with access to composted manure piles. Their diverse dietary habits make them particularly vulnerable to various parasitic infections, especially gastrointestinal parasites (Singh *et al*., 2023).

Host related factors: All poultry species are vulnerable to nematode infection, but young birds under 12 weeks of age typically experience the more severe damage. Younger birds, such as chicks and pullets, are particularly susceptible to high-density infections, whereas older, more resistant birds are less affected (Gualy *et al*., 2005). This susceptibility can be attributed to the gradual development of acquired immunity over time and variations in feeding behaviour and stress susceptibility (Gauly *et al*., 2007; Das and Gauly, 2014; Agmas and Melaku, 2022). Furthermore, it has been established that hens experiencing higher levels of stress or fear exhibit higher parasite excreta egg counts, indicating that stress can affect immune system function and subsequently influence infection intensity (Sherwin *et al*., 2013).

Environment and management related factors: During periods of increased rainfall, there is a rise in the population of intermediate hosts, resulting in a higher prevalence of parasites that rely on these hosts (Tsegaye and Miretie, 2021). Research shows that when temperatures and moisture levels are optimal, nematode eggs can develop into infective larvae more quickly, facilitating their spread (Höglund *et al*., 2011). Ascarid eggs (Ascarida/Heterakis) have a more resistant shell structure, allowing them to exist longer in the environment and still feasible in a wider range of conditions while eggs in capillaria species are more sensitive to climatic conditions such as temperature and less existence in the environment, reducing their transmission potential (Taylor *et al*., 2007).

The occurrence of parasitic diseases is directly connected with production system applied for the animals. There is evidence that different production systems bear different risks of parasite infections for animals (Kaufmann *et al*., 2011). Prevalence of helminthiasis is higher in poultry reared under non-cage than in cage production systems (Shifaw *et al*., 2021). Free-range and backyard flocks are prone to intestinal parasites owing to their scavenging nature and free-range lifestyle (Mlondo *et al*., 2022). On the other hand, unhygienic condition and environment are the most

favourable conditions to support worm populations. Farms with poor hygiene regular health monitoring can lead to higher infections rate (Abdelqader *et al*., 2008).

Climate change might greatly affect gastrointestinal nematode epidemiology in chickens through various mechanisms (Magwisha *et al*., 2002). Changes in precipitation patterns and rising temperatures can hinder the survival, development, and transmission of GIN larvae in the environment (Höglund *et al*., 2011). Increasing temperatures can accelerate egg and larval development of GIN, resulting in shorter generation periods and higher worm loads in chickens (Short *et al*., 2017). Also, increased rainfall has the potential to enhance dispersal and survival of GIN larvae on pasture and, therefore, increase the risk of infection for grazing animals (Fox *et al*., 2012). The changes Absolute binomial dynamic factors in the production systems and in the weather can alter parasites population dynamics which can accumulate the importance of nematode infections in the future (Höglund *et al*., 2012; Tarbiat *et al*., 2020).

2.3. Pathogenesis and Clinical Signs

In chicken, nematodes infection is recognized generally as subclinical, along with profound pathological lesions in the host intestines (Butt *et al*., 2021). Heavy infections of *A.galii* cause enteritis, diarrhoea, weight loss, and reduction in egg production (Dahl *et al*., 2002). A principal pathological lesion in the intestinal tracts of chicken is primarily caused by the larval stages (Magwisha *et al*., 2002). The larval migration causes inflammation, haemorrhage, and malabsorption, while the adults can cause intestinal blockage (Torres *et al*., 2019). *H. gallinarum* is less pathogenic but serves as a vector for *Histomonas meleagridis*, the causative agent of blackhead disease (McDougald, 2005). Heavy infections lead to mild inflammation and cecal wall thickening (Kaufmann *et al*., 2011; Ola-Fadunsin *et al*., 2019). Capillaria infections lead to severe inflammation, necrosis, and haemorrhage and induce weight loss, anemia, and death in heavy infections (Poulsen *et al*., 2000).

2.4. Diagnosis

Accurate identification of parasites is essential for the proper diagnosis and control of the disease. Classical diagnosis-methods including macroscopic diagnosis and microscopic diagnosis have remained the gold standard (Chauhan and Roy, 2007).

Recent advances in the diagnostic methodology have drastically enhanced diagnostic efficiency for nematode infections in poultry (Das *et al*., 2017; Smith and Doe, 2023). Quantitative techniques such as the Stoll dilution method, the universal McMaster method, the Wisconsin flotation method and Mini-FLOTAC(MF) techniques (Rinaldi *et al*., 2022) are routinely used for quantitative FEC estimation in epidemiological studies and for the evaluation of anthelmintic efficacy/resistance by the faecal egg count reduction test (FECRT) (Charlier *et al*., 2022).

2.4.1. Modified Wisconsin sugar flotation method

This method is used for determining the egg per gram of feces and it is probably the most commonly used method. It is the most accurate as it counts all the eggs in 3 gram of feces and it gives quantitative result for counting purpose (Demelash *et al*., 2016). The procedure with centrifugal fecal flotation technique more efficiently recovers parasite eggs and cysts and it requires less time than simple flotation. Centrifugation is especially necessary when the flotation procedure is performed using viscous solution like Sheather's sucrose. It is the most reliable method of recovering ova and cyst of intestinal parasites, including tapeworm and whipworm (Zajac *et al*., 2002).

2.4.2. McMaster technique

It is used to determine the number of EPG of feces. The McMaster technique is one of universally used for FEC. The nematode and cestodes eggs float to immediately below the upper glass of the chamber where they can be readily counted under the microscope, while most fecal debris sinks to the bottom (Demelash *et al*., 2016). Modified McMaster chamber has been developed for trematode eggs and nematode larvae. Some laboratory factors; however, affect the reliability of the McMaster technique; among these, the McMaster slide area or volume is very important. The higher the volume is the higher the reliability of the McMaster technique (Rinaldi *et al*., 2011).

2.4.3. FECPACK methods

FECPAK is one FEC method that was developed in New Zealand in the 1990s (Bosco *et al*., 2014). The original method, FECPAKG1 (G1), was intended to develop an easy-to-use FEC protocol that could be applied on site by the animal owners

themselves and more precisely, farmers first. A key feature of the G1 that facilitated this was the removal of the need for a centrifuge. On-farm monitoring allows anthelmintic treatment to be targeted at heavily infected animals, reducing the development of resistant helminths. G1 has a larger slide volume than the McMaster chamber (1ml vs. 0.3ml) to improve the lower limit of detection, which ranges from 20-35 EPG, depending on the host species and protocol (Godber *et al*., 2015).

The second FECPAK type is FECPAKG2, an internet remote parasitological diagnostic system used in veterinary practice. The initial FECPAK was implemented for nematode egg counting of sheep fecal smears (Rashid *et al*., 2018). FECPAKG2 is based on the metaphor of flotation-dilution, similar to the McMaster technique. Its novelty lies in the condensation of parasite eggs into one observation space in a liquid meniscus. A photograph of the fecal sample is captured, stored offline on a computer, and cloud-synced upon internet access, such that specialists all over the globe can interpret the image at any time (Moser *et al*., 2018).

2.4.4. Mini FLOTAC technique

Mini-FLOTAC technique is a new and efficient method applied in the diagnosis and monitoring of gastrointestinal nematode infections in animals (Cringoli *et al*., 2010). The technique is anticipated to combine advanced technology with high sensitivity, cost-effectiveness, and applicability in diagnosis where intestinal parasitic infection is common in resource-limited settings (Noel *et al*., 2017). It is an adaptation of the FLOTAC method that does not involve the use of a centrifuge. The method applies passive flotation of parasitic forms and can be utilized for the simultaneous diagnosis of helminth eggs/larvae as well as protozoan oocysts (Alowanou *et al*., 2021). This has an advantage over other copromicroscopic techniques, such as the Kato-Katz method, which is unsuitable for larval diagnosis, and the McMaster method, which does not allow intestinal protozoa and larvae to be identified. MF has logistical advantages of having no centrifugation and no need for expensive machinery (Cringoli *et al*., 2017). Worth mentioning is that the larger chamber volume of the MF device could enhance the flotation of nematode eggs, particularly lighter ones and those with thin shells (Barda *et al*., 2014).

2.4.5. Post mortem examinations

The post-mortem diagnosis of infection is commonly utilized in parasitology to determine the number of nematodes present in the gastrointestinal tract for epidemiological studies, assess the intensity of infection, and evaluate anthelmintic efficacy (Modrý *et al*., 2017). For instance, confirmation of adult Ascaridia worms requires post-mortem examination, during which the large white worms are identified during histopathological examination of infected tissues and subsamples infections intensity (Roeber *et al*., 2013; Shifaw *et al*., 2023). The presence of nematodes can be confirmed by observing adult parasites and identifying their larvae through direct examination of intestinal contents (Bello *et al*., 2012). The confirmation of adult Ascaridia worms requires post-mortem examination to identify large white worms through histopathological examination of infected tissues (Shifaw *et al*., 2023).

2.4.6. Serological technique

Serodiagnosis is widely used to diagnose infections caused by tissue-invading parasites. Among the serological tests, the enzyme-linked immunosorbent assay is the most commonly used, and various antigens, are used to detect specific IgGs (immunoglobin Gs) against parasite antigens in the sera or cerebrospinal fluid of patients (Choi, 2024). Serological tests have the disadvantage of being unable to differentiate between past and current infections, and the possibility of cross-reactivity requires careful interpretation. It is important to note that the results of serological tests are not necessarily conclusive and should be interpreted in the context of other symptoms, as well as clinical and imaging findings (Wilkins, 2014).

2.4.7. Molecular techniques

Molecular diagnostic techniques have revolutionized the detection of avian pathogens by providing rapid, sensitive, and specific results in comparison to conventional methods (Tarbiat *et al*., 2021). Polymerase chain reaction is a widely used molecular technique for detecting specific DNA sequences. It serves as an alternative approach, as detect specific antibody responses against antigens in blood before the presence of eggs in faeces (Biswas *et al*., 2021).

Table 4: Characteristics and limitations of different copromicroscopic techniques

Techniques	Diagnostic performance			Technical performance			Main limitations
	Sensitivity	Accuracy	Precision	Cost	Time	Equipment needs	
Basic smear	Very low	Very low	Very low	Cheap	fast	Basic laboratory equipment	Gives positive result only if high parasitism
Simple tube floatation	Very low	Very	Very low	Cheap	long	Basic laboratory equipment	Only used for qualitative
Wisconsin	Low	Low	Very low	Cheap	long	Fully equipped	Lack of precision
McMaster	Medium	Low	Low	Expen sive	Medium	Basic laboratory equipment	Diagnosis of intestinal protozoa limited to coccidia
FECPACK	Medium	Low	Low	Very expen sive	Long	All equipment is provided by manufacture	Utilities is limited to gastrointestinal strongyle
Kato-katz	Medium	Medium	Low	Inexp ensive	Low	Basic laboratory equipment	Require fresh feces
Formalin ether concentration	High for intestinal protozoa only	High for intestinal protozoa only	Very low	Expen sive	Very long	Fully equipped laboratories	Use of formalin and ether, which is hazardous to human and environments
FLOTAC	Very high	Very high	Very high	Cheap	Long	Fully equipped laboratories	Require centrifugation
Min-FLOTAC	High	High	High	Expen sive	Medium	Basic laboratory equipment	trematode needs centrifugation

2.5.Treatments

The treatment of nematode infections in domestic chickens typically involves the administration of anthelmintic drugs. These drugs are specially designed to kill nematodes in the chicken´s intestine. Typical anthelmintic drugs used in the treatment of those infections with nematodes are benzimidazoles (BMZ) including flubendazole, fenbendazole, and albendazole. In addition, piperazine, levamisole, ivermectin were used to limit nematode incidence as to reduce their impact on poultry flocks

(Zirintunda *et al*., 2022). In Australia, prior to 2020 piperazine (PIP) and levamisole were the only registered anthelmintics for poultry with medication provided through the drinking system (Feyera *et al*., 2021). *A. galli* infections can be effectively treated with PIP drug available in different salts for administration in feed or drinking water (Singh *et al*., 2023). Tetramisole, a broad spectrum anthelmintic has been reported to be highly effective against Heterakiasis in chicken. Moreover, fenbendazole and levamisole have been shown to possess anthelmintic activity against *H. gallinarum*, the most frequent helminth species of domestic chicken in Iran, with an efficacy of 83.7% and 71.8%, respectively (Ebrahimi *et al*., 2014; Soudkolaei *et al*., 2021).

2.5.1. Anthelmintic resistance (AR)

Several drugs, including benzimidazoles, imidazothiazoles and macrocyclic lactones, have been shown to be effective against nematodes. However, regardless of the choice of drug, a restrictive, well-considered approach should be taken, as there is a risk of AR development if these drugs are used unwisely (Hoglund *et al*., 2023). In recent years, AR has been a prevalent problem in the control of gastrointestinal nematodes of poultry in virtually every region of the world (Feyera *et al*., 2022). Ascarids resistance to BMZ like fenbendazole have been reported in commercial layer subjected to repeated treatments (Tarbiat *et al*., 2015). Fenbendazole did not affect capillaria spp, showed most commonly resistant nematodes (Soudkolaei *et al*., 2021). A study conducted in Kenya, to evaluate the efficacy of piperazine, levamisole and albendazole against some nematodes of chicken, demonstrated that piperazine citrate was effective against ascarids but had no effects on other worms. It was also found that albendazole was 100% effective against the roundworms and the cestodes (Chege *et al*., 2017). Albendazole is both safe and effective in the treatment of birds for these helminths that reductions in worm burdens from control levels were seen for adult and larval stages of *A. galli*, *H. gallinarum*, and *C. obsignata* ((Tucker *et al*.,2007).

2.6. Prevention and Control

High levels of mortality risk associated with gastrointestinal worms are major concerns in the poultry industry (Höglund *et al*., 2010). Poultry productivity can be improved through the application of sound principles of health protection and preventions. This includes, good management practices, biosecurity measures, pasture

rotation for free range chickens, regular monitoring and strategic deworming practices (Katoch *et al*., 2012; Jilo *et al*., 2022). A strict set of sanitation practices and a clean poultry houses is required to minimise environmental contaminations (Abdullah *et al*., 2021). Developing vaccines for nematode parasites is challenging. This is because the parasite can exist in many different life stages within the host, and it lives in tissues that are less accessible to immune effector cells, such as the intestinal lumen and mucosa. Furthermore, it has been reported that there is no inherent protective maternal immunity against chicken nematodes (Rahimian *et al*., 2017).

3. MATERIAL AND METHODS

3.1. Description of Study Area

This study was conducted in Bishoftu, city of the East Shoa zone, Central Oromia, Ethiopia, from November 2024 to June 2025. Study area is located 47 km southeast of Addis Ababa at latitude 9° N and longitude 40° E and it lies at an altitude of 1,850 meters above sea level. There is a bimodal rainfall pattern in the town, with the long rains starting from June to September, short rains from March to May, and dry seasons starting from October to February, with an average annual rainfall of 866 mm. The mean annual minimum and maximum temperatures are 12.3°C and 27.7°C, respectively, with an overall mean of 18.7°C and yet representative agro-ecologies of the country. The mean relative humidity is 61.3 % (Anon, 2011b). Farmers in the vicinity of the study area use a mixed crop and livestock farming system (Demeke *et al*., 2023).

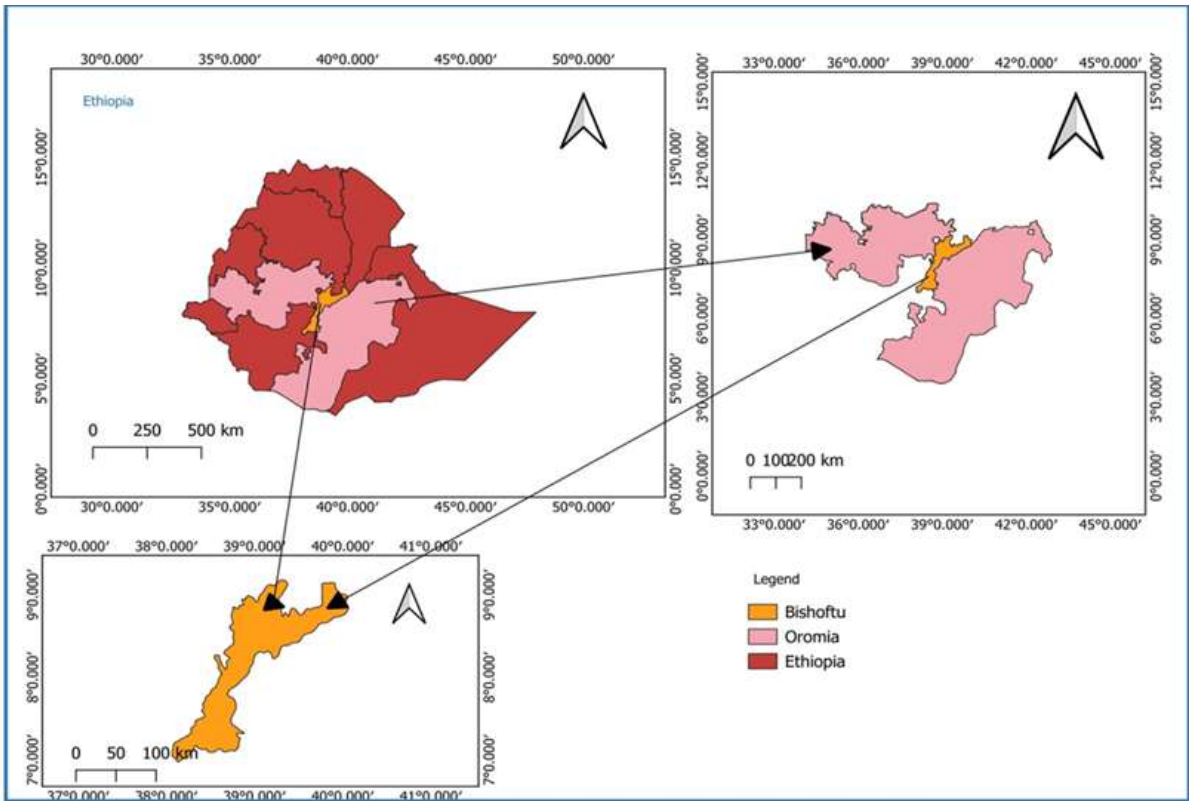

Figure 3: Map of the study areas prepared using QGIS 3.40.6

3.2. Study Design and Sampling Methods

A cross-sectional study design was used for this study from November, 2024 to June, 2025. Bishoftu was selected purposively due to its large number of poultry farms and its role as major poultry productions in Ethiopia. A random sampling technique was used to select the farms. For backyards poultry keeping households, the initial stage involved selecting two kebeles through simple random sampling using lottery methods. In the second stage, all lists of chicken rearing households in each of selected kebeles compiled through Bishoftu city agricultural office and a short pre-survey of field investigations. From this lists the required numbers of households was selected using lottery methods. The sample was proportionally allocated for each kebeles based on the numbers of chicken rearing households. Flock sizes ranged from 50 to 18,000 chickens and categorized as small-scale (50–1,000 chickens), medium-scale (1,001–10,000 chickens), and large-scale (over 10,000 chickens) (Wondmeneh *et al*., 2017). The commercial farms utilized as intensive management system whereas samples from backyards households representing an extensive management system. Sampling was done at the middle of production for layers and at the end of production for broilers

3.3. Sample Size Determination

The sample size was determined using the formula proposed by Thrusfield *et al*. (2018) for simple random sampling of finite populations: $n = [Z^2 \times P \times (1 - P)] / [d^2 + ((Z^2 \times P \times (1 - P)) / N)]$. Where, (n) represents required sample size, (Z) denoted desired confidence level, Pexp, is the expected prevalence, (N) refers to numbers of farms, and d, signifies the precision. Considering finite populations of poultry farms in the districts, the sample size was adjusted accordingly. An expected prevalence of 94% (Shawu *et al*., 2019) for intestinal nematodes and 5% absolute precision, 500 numbers of farms were used to estimate the minimum sample size required. By substituting the value in the above formula, the sample size was calculated to be 75. However, 210 flocks were sampled.

3.4. Sample Collections and Transportations

A pooled fecal sample was collected from 140 intensive and 70 backyard poultry farms for fecal egg counts. Flock farms were visited early morning for sample

collection. Production types, farm scale, breed, and management systems of the farms were recorded as test variables. Approximately 20-30 drops fecal samples, comprising both droppings and freshly voided feces were randomly collected in sample collection container from chicken farms. These were carefully collected from the flock house and the top of freshly dropped feces, excluding soil contamination after wearing disposable plastic gloves. The collected samples were, properly labeled with the necessary information's, and placed in ice box. The fecal samples were transported to the Parasitology Laboratory at Addis Ababa University College of Veterinary Medicine and Agriculture (AAU-CVMA), Bishoftu Ethiopia using an ice box. The samples were analyzed immediately; however, when analysis was delayed, they were stored at a refrigerated temperature of +4°C and analyzed within 48 hours.

Table 5: Data based on farm types, and farm scale

Management	**Types of farm**			**Farm size**			**Total**
	Layers	Dual purpose	Broilers	Large scale	Medium scale	Small scale	
Intensive	80	20	40	6	84	50	**140**
Backyard	40	30	-	-	-	70	**70**
Total	**120**	**50**	**40**	**6**	**84**	**120**	**210**

3.5.Experimental Study

Experimental study was conducted on 42 layers purchased from Addis Ababa University College of Veterinary Medicine and Agriculture poultry farm. A randomized block design was employed where experimental units are first organized into homogeneous blocks based on their fecal egg counts. The chickens were housed in controlled conditions within the experimental research housing facilities at the CVMA. The housing was designed to provide optimal temperature, ventilation, and lighting to ensure the chickens' comfort and minimize stress, which is essential for the accuracy of the study. Each chicken was provided clean water and commercial poultry feed specifically formulated for laying hens to meet their nutritional needs and support their overall health during the trial. The hens were undergo a 7-day acclimatization period before the commencement of the study, allowing them to adjust to the environment while being monitored daily for drinking water and feed consumption as well as any signs of health issues, such as abnormal behavior, lethargy, and changes in

feeding and drinking patterns. All chickens were monitored at a minimum of twice daily for health and well-being.

3.5.1. Treatment protocol

Forty-two hens were divided into three groups of 14 hens each (Table 6). Grouping was done immediately following fecal egg counting after the 7-day acclimatization period. The first group receives no treatment and act as the control group. The second group was treated with fenbendazole, and the third group with piperazine. These treatments were administered through drinking water, in line with the standard treatment guideline for each respective anthelmintic to ensure that all hens receive the appropriate dose of the drugs. The anthelmintic dose was offered in 60 % of normal daily water consumption during the acclimatization period for a period of 8 daylight hours. The medication was administered via nipple drinkers with dose calculation based on total body weight of all hens in the specific group. Hens were withheld from drinking water overnight and anthelmintics were applied the following morning for 8 h in drinking water. At the end of the 8 -h treatment periods, when the total amount of medicated water offered is consumed, medication tanks were turned off and untreated drinking water provided to the experimental hens. In all cases of reconstitution in water, mixtures were stirred until a homogeneous solution or suspension is formed. Fecal samples were collected on day 7 and 14 post-treatment and analyzed for eggs per gram (EPG) using the mini-FLOTAC technique. Then postmortem examinations were conducted on all hens to identify and count worms in the gastrointestinal tract.

Table 6: Experimental groups and treatment dosage regimens

Treatment group	**Number of chicken**	**Total weight (Kg)**	**Recommended dose**	**Mode of application**	**Preparation**
Control group	14	20.3	No treatment	-	-
Gallifen 200mg FBZ/ml, suspension	14	20.1	1mg/kg(0.005 ml/Kg) for 5 consecutive days	Group drinking water	0.005ml FBZ*total body weight of the group in 1 L of drinking water for 5 days
Piperazine citrate 100%, powder	14	20.8	100mg/Kg for 2 consecutive days	Group drinking water	100mg PIP*total body weight of the group in 1L of drinking water for 2 days

3.5.2. Sample collection and handling

For each treatment groups fecal samples were collected from the individual chicken placed in separate cages after observation of freshly excreted feces. Samples were placed in clean, dry plastic tubes with the help of disposable gloves. Representative sampling involved taking feces from each of the chicken in the treatment and control groups on Day 0 (before the treatment) and Days 7 and 14 after the treatment as per the fecal egg count reduction test. All the samples were labeled with a unique identification code, date of collection, and treatment group. At the end of the trial, a subset of chickens from each group was humanely euthanized according to ethics guidelines, and intestines were dissected out at necropsy for counting of adult worms. The entire small intestine and ceca were dissected out carefully and placed in labeled plastic bags. Samples were kept moistened with physiological saline to prevent desiccation of adult worms. Intestinal contents were washed through sieves and examined with a dissecting microscope for recovery and counting of adult nematodes.

3.6. Study Methodologies

3.6.1. Copromicroscopy: fecal egg counts (EPG analysis)

To count the eggs per gram of feces, fecal samples were analyzed using Mini FLOTAC method (Cringoli *et al*., 2017). This method is more sensitive for accurate detection and quantifications of parasite eggs even at low EPG levels compared to the traditional McMaster method (Das *et al*., 2020, Alowanou *et al*., 2021, Shifaw *et al*., 2021b). In brief, a floatation solution was prepared by dissolving 200 g of sugar and 325 g of NaCl in 1000 ml of water (Specific gravity = 1.24). 5 g of pooled fresh feces is weighed into the Fill-FLOTAC container, followed by the addition of 45 ml of flotation solution. Then the sample is thoroughly homogenized using the homogenizer stick of the Fill-FLOTAC. The fecal suspension was filtered through the Fill-FLOTAC and subsequently added to both chambers of the Mini-FLOTAC device. After 10 minutes, the top part of the flotation chambers was translated and the number of worm eggs was counted in each of the two chambers using a light microscope at a 10x magnification. The EPG for each sample is obtained by multiplying the total number of eggs by 25. Although there are some morphological differences between the eggs of *A. galli* and *H. gallinarum* (Zloch *et al*., 2021), they are not easily distinguishable based on morphology alone. Therefore, in this study these eggs are

counted together as ascarid eggs, following the approach described by Thapa *et al*. (2015) and Daş *et al*. (2020). Capillaria eggs are, however, distinct and may be easily differentiated from other parasites' eggs (Merk, 2011).

3.6.2. Worm counts

To identify the types of nematodes and quantify the infections through worm counts, post-mortem examinations were employed following the protocols of the World Association for the Advancement of Veterinary Parasitology (Yazwinski *et al*., 2003). For this purpose, live hens (n=50 from 10 flocks, 5 hens per farm) were purchased from volunteer farms while fresh intact gastrointestinal tracts of broilers (n= 40) and dual-purpose chickens (n=25) were obtained from the Alema poultry abattoir and chickens slaughtered at farm level. For examination, intestines and cecum obtained from all chickens were opened along their entire length using scissors. The small intestinal contents were immediately processed while the cecum along its contents was refrigerated at +4°C for later examination within 24 hours. Contents of each section of the intestine and both caecal tubes were sieved with a 100 μm mesh sieve, flushed with tap water and all visible worms on the sieve were collected and counted. The remaining samples in a Petri dish were examined under a stereomicroscope (40×) to count immature and microscopic worms. All procedures were conducted at a post-mortem facility in the College of Veterinary Medicine and Agriculture, Addis Ababa University.

3.6.3. Evaluation of anthelmintic drug efficacy

The efficacy of fenbendazole and piperazine against intestinal nematodes in chicken was evaluated using fecal eggs count reduction tests. For this purpose, sampling was done at day 0, day 7 and day 14 post treatment. Fecal egg counts were determined using Mini-FLOATAC techniques, and percentages of fecal egg count reductions was calculated following the guideline proposed by Coles *et al*. (1992) to assess anthelmintic efficacy. Then, efficacy of anthelmintics was evaluated by complete reduction of parasite eggs in the treated chicken and Percentage effectiveness against each treatment was determined using the above formula. The means of EPG for each treatment group were used to calculate the percentage efficacy of the anthelmintics. Effectiveness thresholds (Percentage efficacies) for the different anthelmintics were

considered effective above ≥95 %, resistance suspected at FECRT<95% or LCL≥90% and resistance confirmed at FECRT <95% or LCL<90%.

3.7. Data Managements and Analysis

All statistical analyses were performed using JMP®16 (SAS Institute Inc., Cary, NC, USA). Descriptive statistical analysis was used to summarize and present the data. The prevalence of ascarid eggs was calculated as the proportion of the farm examined infected with an ascarid parasites during the study. The association between the prevalence of the disease and hypothesized risk factors was assessed using P-values and the odds ratio for risk factors was computed using logistic regression. Mean worm burden and EPG were tested and analysis. Worm count and EPG were transformed by cube root for statistical analysis. One-way analysis of variance was used to analyse transformed worm count and EPG of all exposure variables. The Tukey HSD test was used to compare mean EPG and worm burden between the exposure variables. Statistical significance was determined by using a P-value of ($P \leq 0.05$) with all analyses meeting the 95% confidence level. The efficacy of anthelmintics was evaluated based on the reduction in FECR%. The arithmetic means of pre-and post-treatment fecal nematode egg outputs and 95% upper and lower confidence limits for the reduction were calculated. Anthelmintic resistance is declared only when the FECR% is less than 95% and the lower 95% CL is less than 90%.

3.8. Ethical Approval

Before sample collection, Ethical approval for this study was obtained from the Ethical Committee of AAU-CVMA (Ref number: VM/ERC/04/17/025, Date: 25/02/2025). The Ethical clearance certificate is attached as evidence. In addition, informal visits were made to the poultry farms to explain the objectives of the study and to obtain informal consent. During sampling, strict biosecurity measures were taken to prevent cross-contamination and minimize the risk of disease transmission between farms.

3.9. Limitations of the Study

One of the main limitations of this study is the difficulty in distinguishing between the eggs of *Ascaridia* and *Heterakis* species during the pre-treatment screening based on fecal egg counts. As the eggs of these two species are morphologically similar, it was being challenging to confirm whether the chickens are harboring *Ascaridia* or *Heterakis* before treatment. The EPG may not fully reflect the actual burden of worms present in the gastrointestinal tract of the infected birds. To address this limitation, the number of chickens per group was increased to at least 14 chickens/per group so that at least 50% of the positive chickens were have *Heterakis* or *Ascaridia* based on prevalence data.

4. RESULTS

4.1. Prevalence and Fecal Egg Counts

Of the 210 studied poultry farms, the overall prevalence of Ascarid eggs (*Ascaridia or Heterakis species*) was 63.8% with the prevalence significantly different among poultry types (P <0.001). Ascarid eggs were detected in 81.7% of layer flocks, 62% of dual-purpose chicken flocks and 12.5% of broiler flocks. The eggs of *Capillaria species* were not detected in any of the sampled farms. The estimated prevalence and distribution of Ascarid parasites across management system, poultry types and farm size are presented in Figure 4.

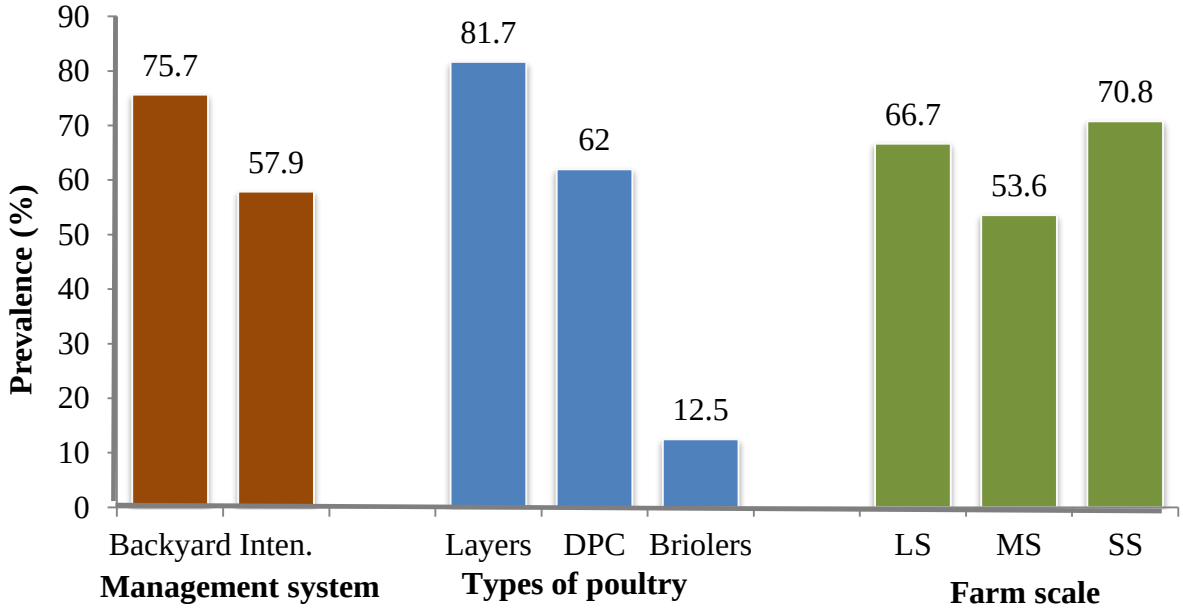

Figure 4: Prevalence of Ascarid parasites based on fecal egg counts

Inten. = intensive; DPC = dual purpose chicken; LS = large scale; MS = medium scale; SS = small scale

The univariate regression analysis for the estimated prevalence of ascarid eggs and associated risks is presented in Table 7. The prevalence of ascarid eggs differed significantly (P <0.05) across poultry types. The highest prevalence was recorded in poultry sampled from layer compared to dual-purpose and broilers chicken flocks. For farm size, the highest prevalence of ascarid eggs was detected in small-scale farms while it was slightly less in medium-scale flocks, but the prevalence did not

significantly vary among the farm size for all of the flock size. The prevalence of ascarid eggs differed significantly (P <0.05) among management systems with higher prevalence were recorded in backyards chicken flocks. The prevalence was nearly equal among the different breeds of the flocks.

Table 7: Prevalence of *Ascaridia*/*Heterakis* species in different exposure variables based on fecal egg counts

Variables	Category	Prevalence	OR (95%CI)	P-value
Management system	Backyard	75.7	Ref	Ref
	Intensive	57.9	0.5 [0.25-0.87]	0.016
Production Type	Broiler	12.5	Ref	Ref
	Dual	62	9.7[3.2-28.8]	0.00
	Layer	81.7	31.2[11-88.7]	0.00
Farm size	Large scale	66.7	Ref	Ref
	Medium scale	53.6	0.6 [0.1-3.2]	0.50
	Small scale	70.8	1.2 [0.2-6.7]	0.86
Breed	Bovans brown	79.4	Ref	Ref
	Cobb500	62	0.04 [0.01-0.12]	0.00
	Dekalb	100	Undefined	
	Local	70	0.7[0.15-2.9]	0.59
	Lohmann brown	83	1.4 [0.5-3.7]	0.5
	Sasso	63	0.4 [0.2-0.96]	0.04

Layer flocks had significantly (P<0.001) higher ascarid burdens (mean EPG = 1105±1586.2, range = 0-7425), compared to dual chicken flocks (Mean EPG = 782±1938.4, range = 0-8000), and broilers (Mean EPG =21±79.4, Range = 0-450). The estimated mean fecal egg counts of ascarid parasites in different poultry flocks and the associated risk factors are presented in Table 8. In this finding, there are no significant differences in the mean EPG across the farm size and management system, while there are significant differences in the mean EPG across poultry types and breeds of the flocks.

Table 8: The fecal egg counts of Ascarids in farms based on the variables.

Variables	Category	Mean EPG±SD	Median	Range	P-value
Managements	Backyards	1108[a]± 2000.8	175	0-8000	0.06
	Intensive	678[a]±1289	213	0-7050	
Farm size	Large scale	675[a]±1241.3	100	0-3150	0.14
	Medium scale	568[a]±1184.9	112.5	0-7050	
	Small scale	1006[a]±1791.5	237.5	0-8000	
Breed	Bovans brown	1400[a]±1793.3	550	0-7050	0.00
	Lohmann brown	853[ab]±1361.3	450	0-7425	
	Cobb500	21[b]±79.4	0	0-450	
	Dekalb	656[ab]±525.3	625	0-1325	
	Sasso	789[ab]±1983.7	100	8000	
	Local	450[ab]±722.6	250	2350	
Poultry types	Layers	1105[a]±1586.2	450	0-7425	0.00
	Dual	782[a]±1938.4	100	0-8000	
	Broilers	21[b]±79.4	0	0-450	

Means with the same letter are not significantly different

4.2. Prevalence and Worm Counts Based on Post-mortem Examination

The findings showed that hens had the highest prevalence of *A. galli* (84%) and *H. gallinarum* (46%) compared to other poultry types (Figure 5). *A.galli* had higher worm with a mean of 11.5±12.3 (range = 0–64) in hens, 12.1±13.4 (range 0–45) in dual-purpose chickens and 1.38±3.4 (range 0–16) in broilers. The mean worm burden for *H. gallinarum* was 5.7±8.3 (range = 0-87) in hens, 9.3±18.3 (range 0–87) in dual-purpose chicken and 0.8±2.8 (range = 0–14) in broilers. The mean worm counts were nearly similar in layer and dual-purpose chicken for both worms, while broilers harbour much lower worm counts.

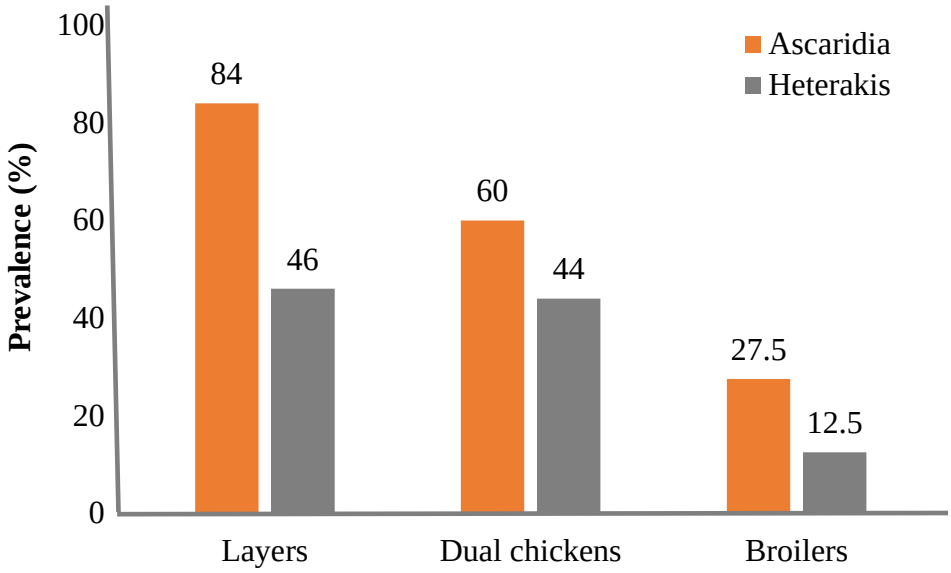

Figure 5: Prevalence of *Ascaridia galli* and *Heterakis gallinarum* based on necropsy

4.3.Anthelmintic Efficacy Test

The present study evaluated the anthelmintic efficacy of fenbendazole and piperazine against intestinal nematodes of chicken using fecal egg count reduction test in a randomized block trails. The mean EPG were recorded on day 0(pre-treatments), day 7, and day 14 post treatments starting from the last day of treatment for each groups. The results are summarized in figure 6. The mean EPG values showed marked reductions in both drugs treated groups compared to control. In the fenbendazole treated groups, the mean EPG dropped sharply from 1625 on day 0(pre-treatments) to 12.5 on day 7, and reach 0 by day 14, indicating complete eliminations of nematode eggs shedding. Similarly, the piperazine –treated group exhibited a decrease in mean EPG from 1407 on day 0 to 39.3 on day 7, and also reached 0 by day 14. The untreated control group, however, demonstrated no significant decline, with mean EPG values remaining high indicative of persistent infection (Figure 6).

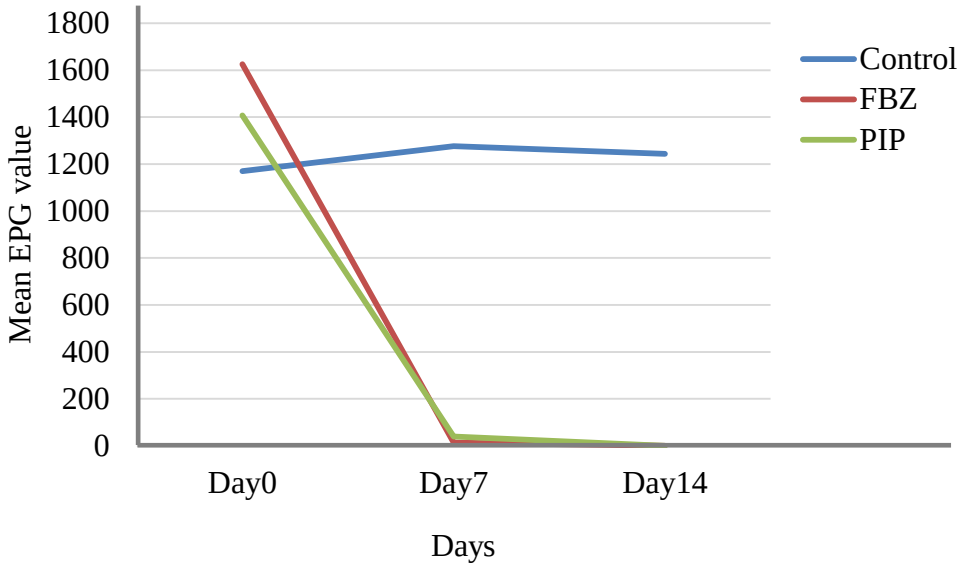

Figure 6: Post-treatment Mean EPG of ascarids in treatment groups

This finding did not showed any indication of reduced anthelmintic susceptibility in intestinal nematodes. Fenbendazole showed a rapid reduction on day 7 with a FECRT of 99.23%, and completely halted eggs shedding by day 14 with 100% FECRT. Similarly, piperazine showed the mean EPG on day 7, representing a FECRT of 97.21% and 100% FECRT on day 14(Table 9). The findings indicate that both drugs are highly efficacious, but fenbendazole has a slightly more rapids onset of actions compared to piperazine.

Table 9: The efficacy status of the tested anthelminthic based on Mean EPG and FECR

Measurements	**Treatment groups**		
	Fenbendazole	**Piperazine**	**Control**
Mean EPG pretreatment(Day 0)	1625	1407	1170
Mean EPG post-treatment(Day 7)	12.5	39.3	1277
Mean EPG post-treatment(Day 14)	0	0	1243
FECRT (Day 7)	99.23%	97.21%	-
FECRT(Day 14)	100%	100%	-
95% UCL at(Day 7)	100%,	99.56%,	-
95%LCL at (Day 7)	97.66%	94.86%	-
Status of efficacy	**Effective**	**Effective**	-

Chickens in the control group harbored 180 *A.galii* and 10 *H.gallinarum*; 14 days post treatment with a total worm count of 190. In contrast, no adult worms were recovered from chickens treated with either fenbendazole or piperazine, indicating complete removal of adult nematodes in the treated groups (Table 10).

Table 10: Adult worm recovery from treatment and control groups

Treatment group	**No of chicken in group**	***A.galii***	***H.gallinarum***	**Total worm count**	**Efficacy**
Control	14	180	10	190	-
Fenbendazole	14	0	0	0	100%
Piperazine	14	0	0	0	100%

5. DISCUSSION

The current study was the first to report the prevalence and distribution of gastrointestinal nematodes in chicken raised in both backyards and commercial farming systems in Ethiopia based on fecal egg counts using Mini FLOTAC and worm counts. The overall prevalence of Ascarid eggs (Ascarida/Heterakis species) was 63.6% in the study. This result is close to the prevalence rates reported by Hussen *et al*. (2012), which was 58%, by Ashenafi and Eshatu. (2004), which was 75.8%, by Wuthijaree *et al*. (2024), which was 72.1%, and by Mlondo *et al*. (2022), which was 66.7%, By Belete and Addis (2015), which was 53.9% in Ethiopia, by Berhanu *et al*. (2014), which was 67.5% in Ethiopia. Furthermore, this finding was consistent with the study conducted in South Africa by Malatji *et al*. (2016), who reported a prevalence rate of 66.44%. In contrast, the current finding was significantly lower than those of reported by Phiri *et al*. (2007) in Zambia, as well as by Kyalo (2012) which reported the rates of 87% and 93.8 % in farm and markets, respectively, in Kenya.

The overall prevalence of the present study was significantly higher than the reports of Tesfaheywet *et al*. (2012) and Baboolal *et al*. (2012) which were 19.01% and 5.5% in South eastern Ethiopia and Trinidad West Indies, respectively. Additionally, the current finding was significantly higher than that of Puttalakshmamma *et al*. (2008), who reported (34.3%) of nematode parasites and that of Wondimu *et al*. (2019), which was 49.5% in Ethiopia. The findings of the current study are also supported by the systematic review and meta-analysis of Asfaw *et al*. (2019) revealing the widespread prevalence of nematode infections in Ethiopian poultry. This prevalence was not surprising, given that the current shift from cage housing system to deep litter system allow chickens direct contact with excreta and soil which dramatically increases the prevalence and burden of helminth (Maurer *et al*., 2009; Shifaw *et al*., 2021a).

The variation in prevalence and intensity of intestinal worm infections across different country can be attributed to differences in management systems, parasite control strategies, and diagnostic techniques employed and sample size. Poor biosecurity such as proximity of poultry farms, poor sanitation, interchange of chicken barns with chickens of different production types, absence of unique clothes for the workers and

visitors, presence of wild birds and inadequate use of appropriate farm protocols (Wossene, 2006; Fulas *et al*., 2018; Tsegaye *et al*., 2023).

The prevalence of ascarid eggs (Ascarida/Heterakis species) based on fecal egg counts was 81.7% in layer flocks, 62% in dual purpose, and 12.5% in broiler chicken flocks which was significantly different among the different poultry types. The prevalence of ascarid eggs in layer flocks in this study was in close agreement with the prevalence reported by Belete and Addis (2015) and Berhe *et al*. (2019) from Bahir Dar town (79.9%) and Mekele (84.4%) in small scale commercial chickens. It also matches the 80% prevalence in conventional free-range layers in Australia, as reported by Shifaw *et al*. (2023). However, it was slightly lower than the 100% prevalence reported by Sherwin *et al*. (2013) in England and Wales. Conversely, it is higher than the 60% by Shiferaw *et al*., 2016, and 49% by Temesgen *et al*., 2024 in Ambo, Ethiopia, in organic free-range (65%) and barn (71.4%) layers in Australia (Shifaw *et al*., 2023), the 30.9% prevalence reported by Thapa *et al*. (2015) in Europe.

The current prevalence of ascarid eggs in broilers (12.5%) was close to the 10.5% prevalence of helminth infections in Trinidad by Baboolal *et al*. (2012). However, it was by far higher than the 0% reported by Kurt and Acici (2008) and Kose *et al*. (2009) in Turkey. Additionally, Rabbi *et al.* (2006) and Afia *et al.* (2019) documented 3.75% and 3.6% prevalence's of helminth infections in broilers in Bangladesh and Nigeria respectively. In contrast, this finding was by far lower than the 98.9% prevalence reported in the United States by Yazwinski *et al.* (2013b). In another study, Singh *et al*. (2021) confirmed that broilers harboured more parasitic infection (40.0%) than layers (35.7%). For the dual-purpose flocks, no comparison data are available as other researches have not disaggregated from other chickens. For instance, Adamu *et al*. (2019) reported pooled helminth infection prevalence without segregation. But it was nearly the 58.0% nematode infection among Ethiopian scavenging chickens (Hussen *et al*. (2012). These show that Ascaridia and Heterakis species have a tremendous impact on the health and productivity of poultry when they infect their intestines. High infection with nematodes may lead to poor feed efficiency, loss of weight, retarded growth, decreased egg production, enhanced susceptibility to secondary infection, and death in extreme cases (Permin and Hansen, 1998, Thapa *et al*., 2015). The economic impact is particularly great for layer flocks, where the birds

are kept indoors for long periods of time, allowing the parasites a longer duration in which to accumulate production losses (Ruff, 1999).

Breed-wise, Bovans brown and Lohmann brown layers presented the highest worm burdens, whereas the EPGs were considerably lower in the case of Cobb500 chickens. This was in line with the report of Kaufmann (2011), who found the higher resistance to *A. galli* infection of local hens than that of Lohmann LSL classic and ISA Brown hens when exposed to an experimental *A. galli* infection. Abdelqader *et al*. (2007) also reported the higher resistance of local chickens than Lohmann LSL white chickens. Local chickens of Jordan infected with *A. galli* revealed significantly fewer worms and excreted less *A. galli* eggs than those of Lohmann LSL white chickens. This pattern could be due to differences in breed susceptibility and production type where layer breeds are kept for a more prolonged period of time and more often raised under less controlled conditions predisposing parasite accumulation (Adamu *et al*., 2019).

Ascarid prevalence was an important factor associated with management system. The current results show that, backyard chickens exhibit a significantly higher prevalence rate of 75.7% compared to intensive management systems. This outcome matches closely with the prevalence rates reported by Shifaw *et al*. (2021a), which was 82.6%; Katoch *et al*. (2012), which was 72.0%; and Abdelqader *et al*. (2008), which was 73.1%. Furthermore, this finding was consistent with the study conducted in Kenya by Mungube *et al*. (2007), who reported a prevalence rate of 74.4%in backyard chickens. Conversely, the current results are noticeably lower than those Phiri *et al*. (2007) in Zambia. These finding reinforce the idea that birds raised in a less protected environment (extensive) are more predisposed to helminth infections because of a greater likelihood of exposure to the infective stages present in the soil, litter and contaminated water sources (Permin *et al*., 1999).

Even though there was no significant variation, a slight difference in the prevalence and intensity of ascarid infections was observed among the farm scale. The highest prevalence and intensity was detected in small-scale farms while it was slightly higher in medium-scale flocks. This was also confirmed by the European research of Rozenn *et al*. (2024) who found that large poultry farms always maintained 81% biosecurity measures. This difference is attributed to varying management practices, deworming, and environmental constraints. Poultry farms on a large scale have relatively good

biosecurity and management as opposed to small and medium-scale. This was also reflected in the biosecurity assessment study of small and medium-scale poultry farms in Ethiopia by Tsegaye *et al*. (2023) who indicated that the biosecurity practices of such farms were under low biosecurity scores below average.

The postmortem worm counts revealed both *A.galli* and *H. gallinarum* are highly prevalent with the prevalence of 84%, 60%, and 27.5% for A. galli and 46%, 44%, and 12.5% for *H. gallinarum* in hens, dual-purpose chickens, and broilers, respectively. Previous studies demonstrated high prevalence rates for these two species ranging from 5-100% for *A. galli* and 2 to 100% for *H. gallinarum* (Abebe *et al*., 1997, Permin *et al*., 1999, Eshetu *et al*., 2001, Rabbi *et al*., 2006, Kaufmann *et al*., 2011, Ogbaje *et al*., 2012, Tesfaheywet *et al*., 2012, Sherwin *et al*.,2013, Belete and Addis, 2015, Ben Slimane, 2016, Idika *et al*., 2016, Das *et al*., 2017, Wokem and Obiyor., 2018, Ybanez *et al*., 2018, Adamu *et al*., 2019, Berhe *et al*., 2019, Ola-Fadunsin *et al*., 2019, Sarba *et al*., 2019, Van *et al*., 2020, Shifaw *et al*., 2021a, Mlondo *et al*., 2022, Ameji *et al*., 2022, El-Debakhyl *et al*., 2024).

The mean worm counts for *A. galli* were 11.5±12.3 in hens, 12.1±13.4 in dual-purpose chickens and 1.38±3.4 in broilers. The mean worm burden for *H. gallinarum* was 5.7±8.3 in hens, 9.3±18.3 in dual-purpose chicken and 0.8±2.8 in broilers. There are few reports on worm burden on commercial poultry farms: Ybanez *et al*. (2018) reported 5.7 worm burden of *H. gallinarum* in hens and 9.3 and 12.1 of *A. galli* and *H. gallinarum* burden in dual-purpose birds. Similarly, Shifaw *et al*. (2023) reported average worm burden of 45.5 and 22 per hen for *H. gallinarum* and *A. galli,* respectively.

In this study, Capillaria species were not detected in any sampled farms. This finding is consistent with other studies in Ethiopia by Worku and Bedane, (2019), Eshetu *et al*. (2001), Tesfaheywet *et al*. (2012) and Wassie *et al*. (2012). Similar trends were also reported in other countries (Rabbi *et al*., 2006, Sherwin *et al*., 2013, Adang *et al*., 2014, Ben Slimane, 2016, Kose, 2009, Permin *et al*., 1999, El-Dakhly *et al*., 2019, Ben Slimane, 2016). However, other studies have reported the occurrence of this worm in various regions of Ethiopia with a prevalence ranging from 1% to 56.3% in intensive poultry and up to 80% in backyard chickens. Specifically, intensive poultry prevalence rates of 1% by Adamu *et al*. (2019), 1.3% by Yadeta *et al*. (2019), 7.3% by

Belete and Addis (2015) and 56.3% by Berhe *et al*. (2019) are reported. In backyard chickens, some of the prevalence documented is 1.6% in central Ethiopia by Ashenafi and Eshetu (2004), 13.1% by Berhanu *et al*. (2014), 5.6% by Sarba *et al*. (2019), and an extremely high 80% by Beruktayet and Mersha (2016) in other parts of the country. On the other hand, Ola-Fadunsin *et al*. (2019) and Wokem and Obiyor (2018) documented significantly low prevalence (0.8%, 2.3%) in intensively managed poultry in Nigeria. Shifaw *et al*. (2021a) recorded the worldwide prevalence of capillaria species as 5.90%.

The results above show that *Capillaria species* are uncommon or rare in certain regions, particularly in farms with good management conditions. This could be attributed to several factors revolving around their transmission dynamics and environmental components. Unlike *Ascaridia and Heterakis*, which have direct life cycles, the transmission of *Capillaria* species needs intermediate hosts, such as earthworms and fish (Merk, 2011) which limits their transmission, particularly under commercial farming systems where these hosts are absent. The second reason is that eggs of *Capillaria species* are more prone to climatic conditions such as temperature and less stable in the environment, which reduces their potential for transmission. Ascarid eggs, on the other hand, possess a more resistant shell structure, which allows them to survive longer in the environment and be viable in a wider range of conditions (Taylor *et al*., 2007). This low prevalence of *capillaria species* in all the farms sampled might suggest variations in the parasite's epidemiology, as well as host specificity or sampling limitations (Maina *et al*., 2017).

The variation in prevalence and intensity of ascarid infection between poultry types could be attributed to management and keeping duration of chickens. Layers are kept for longer durations (typically 18-24 months) compared to broilers (6-8 weeks), with a greater risk of getting infected and reinfected through contaminated water, food, and litter with infective stages (Adamu *et al*., 2019). Similarly, even though they are kept for a shorter period compared to layers, dual-purpose chickens are still kept for longer cycles of production (4-6 months) compared to broilers, allowing more time for parasite development. Furthermore, variation in housing, farm management practices, hygiene measures, and biosecurity measures may also contribute to the observed differences in parasite burdens. Compared to layer and dual-purpose chicken farms,

broiler farms have relatively good biosecurity measures, including good farm hygiene and limited entry of visitors, as supported by our field observations, all of which likely contributed to minimizing parasite transmission. Unlike layers and broilers, which typically receive well-formulated diets, dual-purpose chickens often rely on non-commercial or insufficient feed, which may lead to nutritional deficiencies which further weaken immunity, and increase susceptibility to parasitic infections (Thapa *et al*., 2015;Berhe *et al*.,2019).

The current experimental efficacy trial is the first formal report on the efficacy of commercial anthelmintics against chicken intestinal nematodes in Ethiopia. In this study, we evaluated efficacy of piperazine and fenbendazole against ascarids based on fecal egg reduction test and worm counts. The findings revealed that high efficacy for both drugs, with FECRT values of 99.23% and 100% for fenbendazole, and 97.21% and 100% for piperazine on day 7 and 14, respectively. This high efficacy observed with fenbendazole in this study aligns with previous study reported by Tarbiat *et al*. (2017), which reported a 95% reductions percentages and Yazwinski *et al*. (2009) who found 99.3 to 99.9% of nematicidal efficacies. FBZ attained the desired efficacy as reported by Feyera *et al*. (2022). Similarly, Tarbiat *et al*. (2016b) found efficacy of FBZ against *A. galii*.

Even though AR has not been widely reported for BZs in poultry, studies from the USA have recently documented cases of emerging FBZ resistance in *A. dissimilis* in turkeys (Collins *et al*., 2019) and *H. gallinarum* in chickens (Collins *et al*., 2021). Soudkolaei *et al*. (2021) recorded high prevalence of helminths FBZ resistant, especially *Capillaria* species in free-range layer chickens in Iran. Yazwinski *et al.* (2013b) reported resistance of FBZ with 85.5 and 89.5% effective for the removal of *A. galli* and *H. gallinarum* populations, respectively. Feyera *et al*. (2021) found FBZ administrations in water had low efficacy, with only an 88.7% reduction. Piperazine demonstrated with strong efficacy in current study, with FECRT significantly reduced in treated hens. This is in agreement with earlier studies: Hoque *et al*. (2006) found 100% efficacy of Piperazine against *A. galli* worms on 14th days after treatments. Similarly, Sharif (1980) also found PIP as 100% effective against *A. galli*. Chege *et al*. (2017) studied efficacy of piperazine citrate, in the treatment of chicken naturally infected with gastrointestinal helminths and found 100% efficacy of piperazine during

treatment. However, PIP exhibited reduced efficacy against chicken nematodes as indicated by Feyera *et al*. (2022).

The result of the current study indicates that fenbendazole and piperazine were highly effective (100%) in the removal of adult intestinal nematodes, *A. galli* and *H.gallinarum*, from naturally infected chickens. The complete absence of adult worms were found in treated groups, compared to worm counts in the untreated control group, affirms the therapeutic efficacy of these anthelmintic drugs under controlled circumstances. Adult worm recovery in the control group indicates that active transmission is occurring and that the environment is conducive for survival of these parasites. Successful treatment also indicates no evidence of resistance to fenbendazole or piperazine in the studied population. This could be due to its broad-spectrum activity (fenbendazole) against helminths through the inhibition of microtubule polymerization. Likewise, piperazine is a gamma-amino butyric acid-mimetic drug with flaccid paralysis effect on nematodes that causes their expulsion (Feyera *et al*., 2021).

6. CONCLUSION AND RECOMMENDATIONS

In conclusion, the results of the current study demonstrate that *A. galli* and *H. gallinarum* are highly prevalent in poultry kept under commercial and backyard management systems in Ethiopia. Such heavy infections are likely to adversely affect the health, production performance and welfare of chickens, leading to significant economic consequences. It looks like the major determining factors for the high prevalence and burden are poor management practices and the length of time the chickens are kept. The results of anthelmintic test demonstrated that piperazine and fenbendazole are highly effective at standard dose, achieving the values exceeding percentage reductions. Therefore, based on the above conclusion the following recommendation were forwarded

- Effective control measures such as good management and regular deworming based on infection intensity are urgently needed.
- Awareness creation on the significance of helminth in flocks and effective worm management is crucial for poultry owners.
- It is recommended that the Fecal Egg Count Reduction Test and worm burden assessments should be used routinely to monitor for anthelmintic effectiveness and resistance ahead of time.
- Conduct further research to monitor anthelmintic resistance and identify alternative control strategies.

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8. APPENDICES

Annex 1: Procedure for MiniFLOTAC methods

- Take 5g of fecal sample and add of FS (dilution ratio 1:20) to the Fill-FLOTAC container.
- Homogenize the fecal sample with a wooden spatula, and then fill the conical collector (feces) of the Fill-FLOTAC and level the surface.
- Homogenize the fecal suspension by pumping the conical collector up and down (10 times) in the container while turning it right and left.
- Attach the tip to the lateral hole of the Fill-FLOTAC. Invert the Fill-FLOTAC 5 times to mix and squeeze the container to filter the sample through the lid's filter.
- Pour the sample from the tip into the flotation chambers of the Mini-FLOTAC until a meniscus forms.
- After 10 minutes, use the key to turn the reading disk clockwise (~90°) until it stops, separating the floating PEs from the fecal debris in the Mini-FLOTAC apparatus.
- Place the microscope adaptor on the microscope stage and position the Mini-FLOTAC with the ruled grid of chamber no. 1 on the right. Focus on the grid, starting from one corner, and count the different PEs in all 12 sections of the first chamber using a hand tally counter.
- Repeat the count for the second chamber. Calculate the multiplication factor for EPG by dividing the dilution ratio by the volume (Cringoli *et al*., 2017).

Annex 2: Procedure for Worm Counting

- Euthanize the chickens humanely
- Incise midline abdomen and open up the internal organs, and then carefully remove the gastrointestinal tract.
- Separate the small intestine and place it in a Petri dish or tray containing water/ saline.
- Incise longitudinally in the intestine using blunt-ended scissors and flush out intestinal contents into the dish thoroughly with water.
- Massage gently the mucosal surface with a glass slide or a spatula to dislodge any adherent worms.

- Immediately process the small intestine contents, while refrigerating the cecum and its contents at +4°C for examination within 24 hours.
- Sieve the each section of the intestinal contents and the two cecal tubes through a 100 μm mesh sieve by adding tap water to rinse through the sieve.
- Collect and count all visible worms retained on the sieve (Yazwinski *et al*., 2003).

Annex 3: Types of poultry considered during the study: a) broiler flocks, b) layer flocks c) Sasso

a) broiler

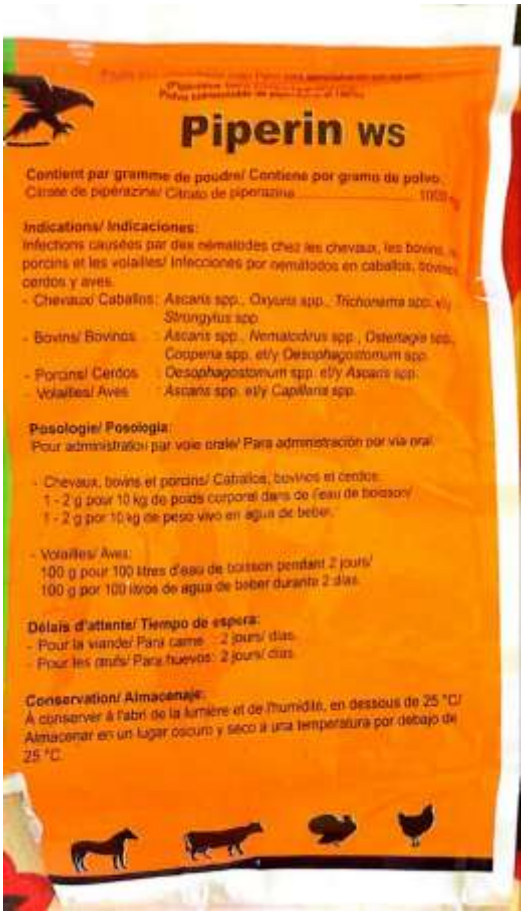
b) laver

c) Sasso

Annex 4: Some pictures during for EPG analysis

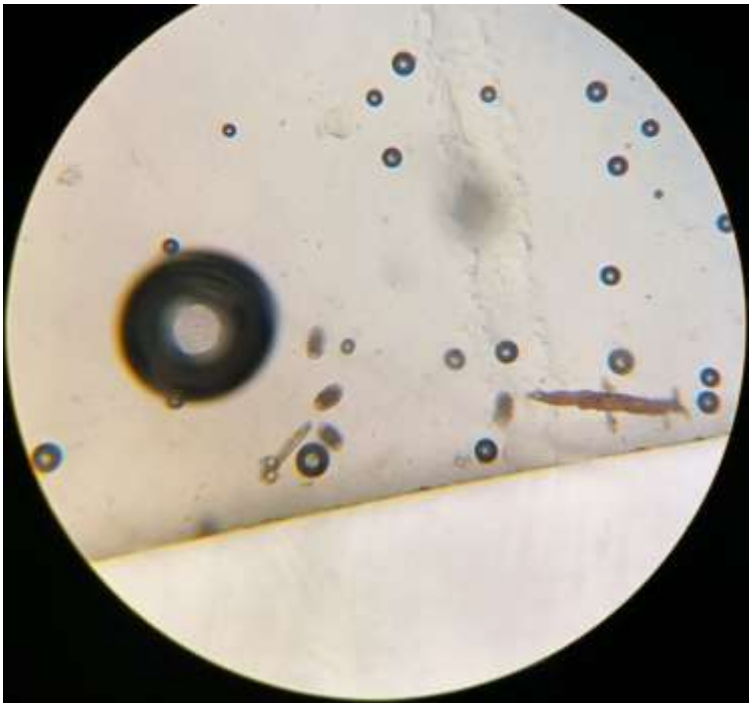
Annex 5: Experimental groups and Chicken facilities

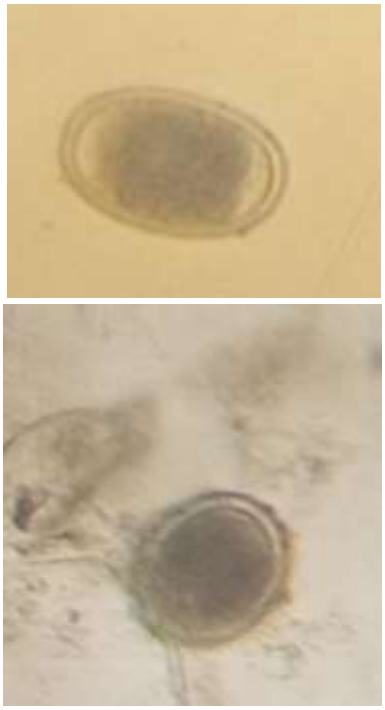
Annex 6: Drugs used for the experimental anthelmintic efficacy



a) Piperazine **b)** Febendazole (Gallifen**)**

Annex 7: Some picture of Ascarids eggs detected in pooled fecal samples



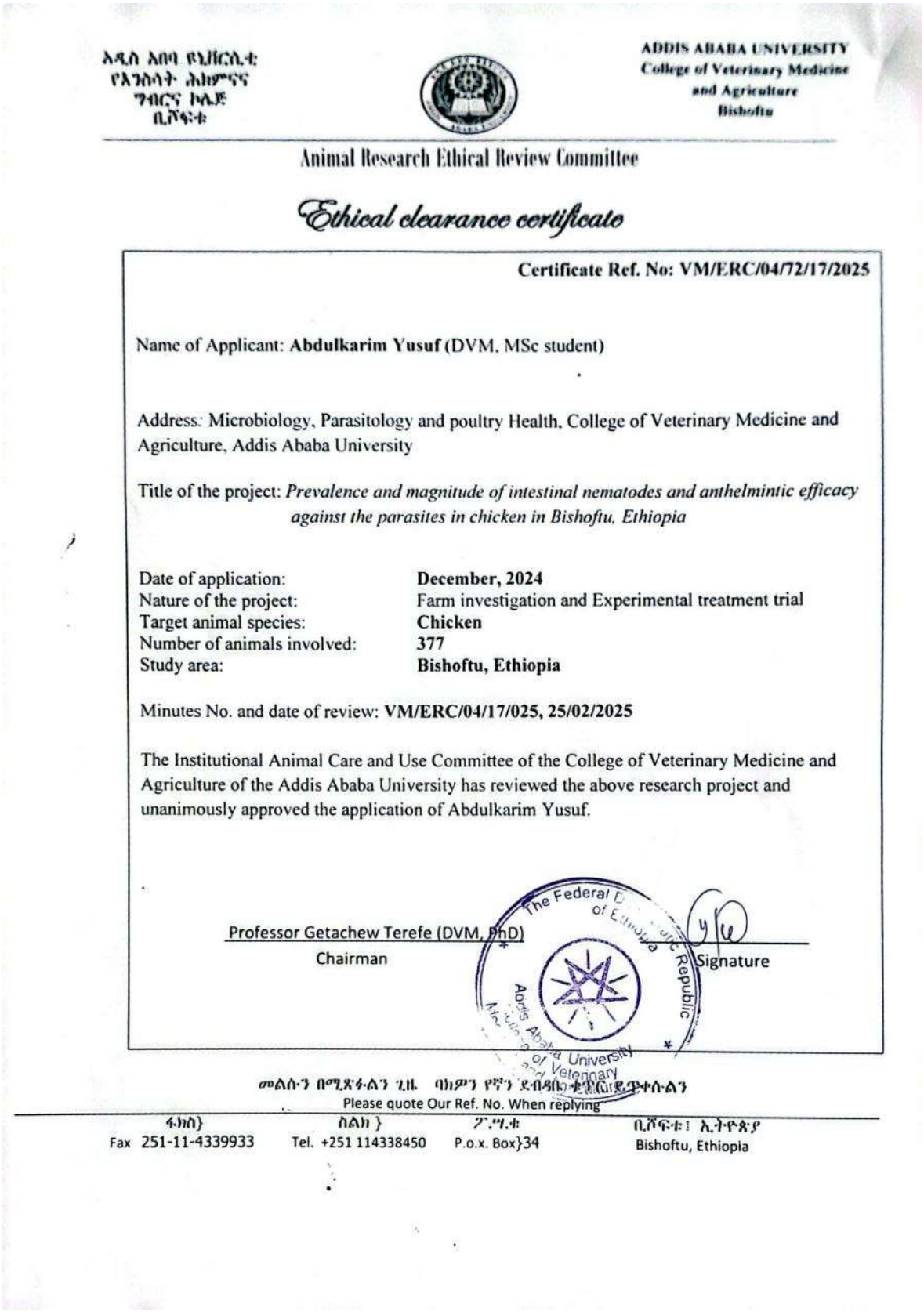


Annex 8: Adult ascarid worms collected from intestine of the chickens

a) A. galli from small intestine

b) *Heterakis gallinarum* from cecum

Annex 9: Ethical Clearance Certificate



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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/04/72/17/2025

Name of Applicant: **Abdulkarim Yusuf** (DVM, MSc student)

Address: Microbiology, Parasitology and poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Prevalence and magnitude of intestinal nematodes and anthelmintic efficacy against the parasites in chicken in Bishoftu, Ethiopia*

Date of application:	**December, 2024**
Nature of the project:	Farm investigation and Experimental treatment trial
Target animal species:	**Chicken**
Number of animals involved:	**377**
Study area:	**Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/17/025, 25/02/2025**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Abdulkarim Yusuf.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

The Federal Democratic Republic of Ethiopia
Addis Ababa University College of Veterinary

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Annex 10: Plagiarism reports

PREVALENCE AND MAGNITUDE OF INTESTINAL NEMATODES AND ANTHELMINTIC EFFICIACY AGAINST THE PARASITES IN CHICKENS IN BISHOFTU, ETHIOPIA

by Abdulkerim Yusuf

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PREVALENCE AND MAGNITUDE OF INTESTINAL NEMATODES AND ANTHELMINTIC EFFICIACY AGAINST THE PARASITES IN CHICKENS IN BISHOFTU, ETHIOPIA

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