

**AKENTEN APPIAH- MENKA UNIVERSITY OF SKILLS TRAINING AND
ENTERPRENEURIAL DEVELOPMENT**

MPHIL THESIS

**EFFICACY OF ANTHELMINTICS AGAINST CANINE HOOKWORM
INFECTIONS IN THE BONO EAST REGION OF GHANA**

SAMUEL AYETIBO OFORI

AUGUST, 2024

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A Thesis submitted to the Department of Biological Sciences Education of the Faculty of Science Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development in partial fulfilment of the requirements for award of a Master of Philosophy degree in Biology

AUGUST, 2024

DECLARATION

Candidate's Declaration

I hereby declare that this dissertation is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Samuel Ayetibo Ofori

Signature: Date:

Supervisors' Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with guidelines on supervision of dissertation laid down by the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development.

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ABSTRACT

Hookworm infections present a major health risk to dogs, especially in areas characterized by warmer climates and poor sanitation. This cross-sectional study was undertaken to determine the prevalence of hookworm infections and the efficacy of anthelmintic treatments in dogs from the Bono East Region of Ghana. Four hundred and ninety-one (491) canine stool samples were examined using the McMaster technique to ascertain the prevalence of hookworms. Using *in vivo* and *in vitro* techniques, the efficacy of three anthelmintics (Albendazole, Pyrantel, and Niclosamide) was assessed in an experimental control trial involving dogs naturally infected with hookworms. The effects of the drugs on hematological and biochemical parameters were measured within a 14-day period to assess changes over time. The study found a total prevalence of 54.2% (266), with significantly higher infection rates in puppies (69.8%, 97), hunting (64.1%, 91) and rural dogs (84.2%, 160). Logistic regression identified age, purpose, and settlement type as risk factors for infection. Of the three treatments, niclosamide was the most efficacious, reducing egg counts by 95%, while albendazole was the least efficacious (-69%). *In vitro* tests confirmed the superior performance of niclosamide, with the lowest IC₅₀ value of 29.19 µg/ml. Hookworm-infected dogs exhibited anemia, eosinophilia, hypoalbuminemia, and hypoproteinemia. There was significant improvement in the haemato-biochemical parameters after treatment, particularly in niclosamide-treated dogs. Veterinarians can consider niclosamide, especially in resource-limited settings, due to its affordability. The findings emphasize the importance of regular monitoring and treatment of hookworm infections to improve the overall health and well-being of dogs in the region. To the best

of my knowledge, this is the first report on anthelmintics resistance of dog-related hookworms in Africa.

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DEDICATION

I dedicate this work to my family for their support, advice, and encouragement throughout my education and research period. I further dedicate this work to all my loved ones who, in one way or another, have contributed to the success of this research piece.

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ABBREVIATIONS

ALT	-	Alanine Transaminase
AST	-	Aspartate transaminase
DMSO	-	Dimethyl Sulfoxide
EDTA	-	Ethylenediaminetetraacetic acid
EHI	-	Egg Hatch Inhibition
EPG	-	Egg Per Gram
FEC	-	Fecal Egg Count
FECRT	-	Fecal Egg Count Reduction Test
Hbg	-	Haemoglobin
HCT	-	Haematocrit
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
MCV	-	Mean Corpuscular Volume
PLT	-	Platelets
RBC	-	Red Blood Cell
SEM	-	Standard Error of the mean
TC	-	Total cholesterol
WAAVP	-	World Association for the Advancement of Veterinary Parasitology
WBC	-	White Blood cells

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Hookworm infections are major health issues for companion animals, particularly dogs and cats, across the globe (Traub et al., 2021), and Ghana is no exception. These infections caused by parasitic nematodes can lead to various pathological consequences and pose a threat to the overall well-being of dogs. Dogs from a number of countries, including Bangladesh (Singh et al., 2022), Australia (Palmer et al., 2007), India (George et al., 2016; Traub et al., 2021), Nigeria (Kamani et al., 2021; Sowemimo, 2012), and Ghana (Amisshah-Reynolds et al., 2016; Boyko et al., 2020; Mawuko et al., 2015a) have been found to have hookworm infections. Indicating the wide distribution of hookworms and the zoonotic threats they pose to humans. The existence of hookworm species like *A. caninum*, which can also infect humans, raises the possibility that dogs could serve as carriers of zoonotic hookworm illnesses (Jimenez Castro et al., 2019; Wongwigkan & Inpankaew, 2020). The high prevalence of hookworms in dogs and the associated public health risks emphasize the importance of effective anthelmintic treatment strategies (Nezami et al., 2023; Walker et al., 2023).

Anthelmintic medications are frequently used to treat and manage hookworm infections. These include Pyrantel pamoate, Ivermectin, Fenbendazole, Milbemycin oxime and Benzimidazole (BZ) anthelmintics such as albendazole, levamisole and mebendazole (Starkey & Blagburn, 2021). These medications have generally been successful in treating

hookworm infections, lowering the parasite burden, and enhancing the general health of dogs that are infected. However, there is increasing concern around the world about hookworms developing anthelmintic resistance. (Nezami et al., 2023; O'Halloran, 2021; Traversa, 2012). When hookworm populations lose their sensitivity to anthelmintic medications or stop responding to them completely, anthelmintic resistance develops. The genetic traits of the parasites, selection pressure from repeated anthelmintic treatments, and environmental factors are some of the causes of this resistance. The genetic traits of the parasites, selection pressure from repeated anthelmintic treatments, and environmental factors are some of the possible causes of this resistance. (Leutenegger et al., 2023). The consequences are severe, including treatment failures, protracted infections, and possible harm to the health of the animals.

Despite anthelmintic treatment, reports of hookworm infections in dogs have persisted in the United States (Jimenez Castro et al., 2019). Resistance has been observed across multiple breeds, with greyhounds being significantly overrepresented (Jimenez Castro et al., 2019). Real-time PCR techniques have made it easier to identify the F167Y polymorphism in *A. caninum*, a genetic marker linked to benzimidazole resistance. (Leutenegger et al., 2023). There have also been reports of genetic BZ resistance indicators in *A. caninum* in Canada, suggesting the emergence and possible spread of resistant hookworms there. (Evason et al., 2023). The issue of anthelmintic resistance is not restricted to North America. According to a study, pyrantel embonate's effectiveness against *A. caninum* significantly decreased in the Pacific region, indicating high levels of anthelmintic resistance. (Kopp et al., 2007). Levamisole's effectiveness has also been

shown to change with age, therefore rigorous adherence to prescribed treatment plans is necessary (Lee et al., 2013).

Studies have evaluated the impact of hookworm infections on canine hemato-biochemical parameters in addition to anthelmintic resistance. (Jarry & Alfatlawi, 2023; Kebbi et al., 2020; Salem et al., 2015). Specifically, haematological alterations such as red blood cell count (RBC), hemoglobin (Hb), packed cell volume (PCV), and eosinophil counts have been linked to *Ancylostoma caninum* infections. (Aref et al., 2018; Jarry & Alfatlawi, 2023; Kebbi et al., 2020; Salem et al., 2015). Similar alterations in serum biochemistry have also been noted in relation to hookworm, including changes in total protein levels, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) (Aref et al., 2018; Nwoha et al., 2013).

In Ghana, the number of domestic dogs has significantly increased. (Mawuko et al., 2015a). A high frequency of hookworm infections in dogs has coincided with this increase. (Amissah-Reynolds et al., 2024). With the global surge in anthelmintic resistance (Jimenez Castro et al., 2019), it has become imperative to assess the situation in Ghana. It is plausible that these dogs are highly exposed to hookworm infections and could be developing resistance to current treatments. Addressing these issues is important for achieving Sustainable Development Goal (SDG) 3, which focuses on healthy lifestyles and promoting well-being. Thus, in the Bono East Region of Ghana, the study aimed to evaluate the effectiveness of anthelmintics against canine hookworm infections.

1.2 Statement of Problem and Justification

In veterinary medicine, the application of anthelmintic drugs has been extensively used to treat hookworm infections in dogs and other domestic animals. However, anthelmintic resistance is a serious issue that is endangering the effectiveness of current treatment methods. (Kopp et al., 2007). In Ghana, little study has been done on the efficacy of anthelmintic resistance, particularly with regard to hookworm infections in domestic animals. Numerous studies carried out in Ghana have evaluated the frequency of hookworm infections in pets (cats and dogs). (Amissah-Reynolds & Ofori, 2023; Amissah-Reynolds et al., 2016; Boyko et al., 2020). Humphries et al. (2013, 2011) report human hookworm infection rates of 45% and 39.1%, respectively, and emphasized the potential development of resistance to albendazole. Although these studies focus on humans, the environmental and socioeconomic factors that lead to hookworm infections in people could similarly affect dogs. The studies highlighted hookworm prevalence but did not cover anthelmintic resistance, revealing a gap in knowledge about resistance patterns.

Concerns regarding possible medication resistance in Ghana have been raised by reports of treatment failures and recurrent hookworm infections in humans. Concerns regarding possible medication resistance in Ghana have been raised by reports of treatment failures and recurrent hookworm infections in humans. (Orr et al., 2019; Humphries et al., 2013, 2011). The effectiveness of albendazole (ALB) in treating hookworm infections appears variable and may be declining, suggesting emerging resistance (Humphries et al., 2013). Recent studies have shown inconsistent reductions in hookworm egg (89% dry and 93% rainy season) counts following ALB treatment (Hamidu et al., 2017). However, the overall

efficacy of the treatment remains suboptimal. Community-level variations in ALB's effectiveness, with cure rates as low as 0% in some areas and an overall cure rate of only 35% in Kintampo, Bono East Region (Humphries et al., 2013).

The presence of anthelmintic resistance in hookworms is also reported in other regions. Studies in Australia, Canada, and the United States have documented decreased efficacy of anthelmintic drugs and the presence of genetic markers associated with resistance (Evason et al., 2023; Venkatesan et al., 2023; Kaplan, Schwenkenbecher 2009 & Kopp et al., 2007). Recent findings from neighbouring West African countries, such as Nigeria (Agube et al., 2023). This emphasize the urgency of investigating this issue in Ghana, given the potential for shared ecological factors and geographical proximity.

Several important aspects of veterinary treatment procedures are highlighted by a study carried out at the Kintampo Municipal Veterinary Clinic in Bono East Region, Ghana. A major In other areas, hookworms have also been found to be resistant to anthelmintics. Research conducted in the US, Canada, and Australia has shown that anthelmintic medications are less effective and that resistance-related genetic markers are present. finding is that over 50% of records lack details like dosage and administration methods of antibiotics. Similar documentation issues with anthelmintics may obscure resistance levels and hinder mitigation efforts. The study revealed an over-reliance on tetracycline, used in 99.6% of cases, suggesting a similar dependence on a limited range of anthelmintics (Adeapena et al., 2021). This raises concerns about increased resistance development. Adeapena et al. (2021) emphasize the need for further research to understand the efficacy

of drug resistance in Ghana's domestic animal population. The efficacy and extent of anthelmintic drug resistance in hookworms among dogs in the Bono East Region of Ghana remain largely unknown. Therefore, the study assesses the efficacy of common anthelmintic drugs used to treat hookworms among dogs in Bono East Region, Ghana.

1.3 Hypothesis

Null Hypothesis (H₀): The prevalence of hookworm infections in dogs in Ghana's Bono East region will be considerably decreased by the use of anthelmintic medications.

Alternative Hypothesis (H_a): The prevalence of hookworm infections in dogs in Ghana's Bono East region will not be considerably decreased by the use of anthelmintic medications.

1.4 Objective of the Study

1.4.1 Main Objective

This study's primary objective was to evaluate the efficacy of anthelmintics in treating dog hookworms in Ghana's Bono East Region.

1.4.2 Specific Objective

Specifically, the study sought to:

1. determine the prevalence of hookworm infections in dogs in Bono East Region.
2. assess the impact of hookworm infection on haematological and serum biochemical parameters in dogs.

3. assess the efficacy of several anthelmintics in treating hookworm infections among dogs in the Bono East Region.

1.5 Significance of the Study

The study's conclusions aid in the creation of evidence-based treatment plans. Veterinarians and other animal health specialists can choose and administer anthelmintic medications with knowledge of the effectiveness of anthelmintic resistance, guaranteeing the best possible treatment results. Information on the possible hazards to human health can be obtained from this study by examining the effectiveness of anthelmintics. In order to protect public health and put appropriate preventive measures into place, this knowledge is essential. Veterinary clinics, the livestock industry, and pet owners all face financial consequences when it comes to effectively managing hookworm infections in dogs. The study's findings highlight a few elements that lead to anthelmintic resistance. The financial impact of poor therapies, such as higher veterinary expenses and lower production, can be lessened by developing interventions. The results of this study can guide the creation of long-term strategies to prevent hookworm infections caused by anthelmintic resistance.

1.6 Organization of the Study

The study is organized into five chapters. Chapter one is the introduction, which provides the study's background, problem statement, objectives, hypothesis and significance. Chapter Two focuses on the literature review, where relevant studies are discussed, including the theoretical and related literature to the objective and gaps in knowledge are identified. Chapter Three describes the research methodology, including the experimental

design, study area, population, sampling techniques, data collection methods, and ethical considerations. Chapter Four presents the results and discusses the findings with the help of tables and charts, linking them to the study objectives and existing literature. Chapter Five concludes the study by summarizing the key findings and providing recommendations for practice, policy, or further research.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Gastrointestinal nematodes (GINs), specifically hookworm species, pose a considerable threat to dogs, resulting in serious clinical disorders such as anaemia and weight loss. The presence of hookworm infections in dogs in the Bono East Region of Ghana presents significant issues for pet owners and public health officials. This literature review explores various aspects of hookworm infections in dogs. This includes the diversity and prevalence of these parasites and the efficacy of different anthelmintic treatments and diagnostic techniques. Also, it addresses the growing issue of anthelmintic resistance and emphasizes the importance of integrated control measures to mitigate the zoonotic risks associated with canine hookworm infections.

2.2 Gastrointestinal Nematodes of Companion Animals

The phylum Nematoda (roundworms) is known for its extensive diversity, with around 30,000 described species and estimates suggesting there could be over a million species globally (Kiontke et al., 2021). Nematodes are among the most abundant animals on Earth, thriving in various environments (Thaenkham et al., 2022).. While many nematodes are free-living, a significant number are parasitic, posing serious health threats to plants, animals, and humans worldwide (Thaenkham et al., 2022) of which dogs and cats.

Animal gastrointestinal nematodes cause various clinical signs and health issues, from mild gastrointestinal disturbances to severe systemic illness. These parasites can lead to weight loss, diarrhoea, anaemia, and even death, significantly affecting the quality of life of pets and incurring substantial veterinary costs for owners (Szewc et al., 2021). The most clinically significant GINs in companion animals include species from the genera *Toxocara*, *Ancylostoma*, *Trichuris*, and *Strongyloides*. These nematodes can cause various health problems in dogs and cats, affecting their gastrointestinal system and sometimes other organs (Rinaldi et al., 2022).

Toxocara canis (dogs) and *Toxocara cati* (cats) are among the most common nematodes infecting companion animals. These nematodes are known for their zoonotic potential, causing visceral and ocular larva migrans in humans, particularly children (Singaravelu et al., 2015). Adult worms reside in the intestines, where they can cause gastrointestinal signs, such as vomiting, diarrhoea, and abdominal discomfort. Heavy infections may lead to intestinal obstruction. (Bharti et al., 2018). *Ancylostoma caninum* (dogs) and *Ancylostoma tubaeforme* (cats) are hookworms that attach to the intestinal mucosa and feed on blood, leading to significant blood loss and anaemia, especially in young animals. Clinical signs include diarrhoea, which may be bloody, weight loss, and lethargy. Severe infections can be fatal if not treated promptly (Shooraj & Mahdavi, 2022a).

The whipworm *Trichuris vulpis* predominantly infects dogs, residing in the cecum and large intestine. Infections can lead to chronic colitis, characterized by diarrhoea, which may be intermittent and contain mucus or blood, weight loss, and dehydration (Traversa, 2012).

Strongyloides stercoralis affects dogs and cats and has a complex life cycle involving free-living and parasitic phases. Clinical manifestations include gastrointestinal disturbances such as diarrhoea and weight loss. In immunocompromised animals, the infection can become severe and disseminated (Wulcan et al., 2019; Page et al., 2018).

Gastrointestinal nematodes of companion animals typically have direct life cycles, although some species may have more complex cycles involving paratenic hosts. The life cycle stages include eggs, multiple larval stages, and adult worms, all of which can be identified through various diagnostic techniques such as faecal flotation and PCR (Hii et al., 2018). Many gastrointestinal nematodes of companion animals are zoonotic, posing a risk to human health. *Toxocara* spp. and *Ancylostoma* spp. are particularly concerning due to their ability to cause significant disease in humans, including visceral and ocular larva migrans and cutaneous larva migrans, respectively (Thaenkham et al., 2022). Public health measures such as regular deworming of pets, proper hygiene, and public education are vital in preventing zoonotic infections. Effective management of GINs in companion animals includes routine veterinary care, regular deworming protocols, and environmental control measures. Owners should be educated on the importance of maintaining hygiene and promptly treating any infections to prevent the spread of these parasites (Butala et al., 2021). Regular deworming should be performed based on the animal's age, lifestyle, and risk of exposure. Puppies and kittens should be dewormed every two weeks until 12 weeks of age, followed by monthly treatments until six months. Preventing contamination of the environment with feces is crucial. Regular cleaning of living areas and proper disposal of feces can reduce the risk of transmission (Alho et al., 2018).

2.3 Hookworm Disease

Necator americanus and *Ancylostoma duodenale* are the two primary nematode parasites that cause hookworm disease, a common parasitic infection in humans. *Ancylostoma ceylanicum*, *Ancylostoma braziliense*, and *Ancylostoma caninum* are further species that infect mammals. (Oliveira-Arbex et al., 2022). The transmission of hookworm infection occurs when the skin comes into contact with larvae present in soil contaminated with human feces. The soil becomes infectious approximately 5-10 days after contamination and remains so for about 3-4 weeks, depending on environmental conditions (George et al., 2015). These parasites reside in the intestinal tract, causing a range of pathological effects including tissue damage and anemia, which is particularly severe in children and pregnant women (Latha, 2022; Shooraj & Mahdavi, 2022a). The contaminated soil remains

When the skin comes into contact with hookworm larvae found in soil tainted with human waste, the infection is spread. Depending on the environment, the soil becomes infectious 5–10 days after contamination and stays that way for 3–4 weeks. When the skin comes into contact with hookworm larvae found in soil tainted with human waste, the infection is spread. Depending on the environment, the soil becomes infectious 5–10 days after contamination and stays that way for 3–4 weeks. When the skin comes into contact with hookworm larvae found in soil tainted with human waste, the infection is spread. Depending on the environment, the soil becomes infectious 5–10 days after contamination and stays that way for 3–4 weeks. According to estimates, there are between 576 and 740 million hookworm infections worldwide. (Riaz et al., 2020).

While many cases are asymptomatic, hookworm infection primarily causes anemia, low levels of albumin in the blood, and malnutrition. In addition, it can lead to intellectual and cognitive impairment as well as stunted growth in children (Orevaoghene et al., 2024). Because the majority of people with mild to moderate hookworm infections do not exhibit symptoms, preventative and control efforts have historically been neglected globally. (Hossain & Bhuiyan, 2016a). Because they have less access to clean water, sanitary facilities, and health education, the poorest people in developing countries have historically been disproportionately afflicted by hookworm infection. (Roach, 2020).

Despite the lack of noticeable symptoms in many cases, hookworm disease significantly contributes to the prevalence of anemia and malnutrition in developing countries (Loukas et al., 2016a). It is especially prevalent in rural tropical and subtropical areas of Latin America, Asia, and sub-Saharan Africa. Treatment for individual hookworm cases involves iron replacement therapy and the use of anthelmintic drugs (Hossain & Bhuiyan, 2016a). However, eradicating hookworms at the community level has proven challenging, even with intensive annual programs implemented in schools. This difficulty may be attributed, in part, to the failure to eliminate the infection in adults with a high worm burden (Bijapore, 2020). Nonetheless, achieving successful control and eradication of hookworms is a worthwhile objective, as it could yield substantial economic and social benefits for large portions of Africa and Asia. School-based deworming programs have been effective in reducing hookworm infections in children, but they may miss positive cases in adults. The goal of creating a vaccine to prevent hookworm is important, and a number of proteins have been investigated as possible targets for the vaccine (Butala et al., 2021). Before an

effective hookworm vaccine is made available, further study is necessary. Metabolic profiling can help us better understand hookworm infection by analyzing how organisms respond metabolically to various stimuli, such as illnesses and physiological changes.

2.4 Life Cycle of Hookworm Parasite

The life cycle of the hookworm parasite in dogs consists of multiple phases, begin with the infective third-stage larvae (L3). The larvae of these organisms enter the host's body either by penetrating the skin or by being ingested. Upon entering, they migrate to the small intestine and develop into mature hookworms (Hawdon & Wise, 2021). Adult hookworms adhere to the lining of the intestines, suck blood, and produce eggs that are expelled in the faeces of the host (Uppal et al., 2017). The eggs undergo hatching in the surrounding environment, which leads to the release of larvae. These larvae then undergo two moulting stages to transform into infective L3, so completing the cycle (Hawdon & Wise, 2021).

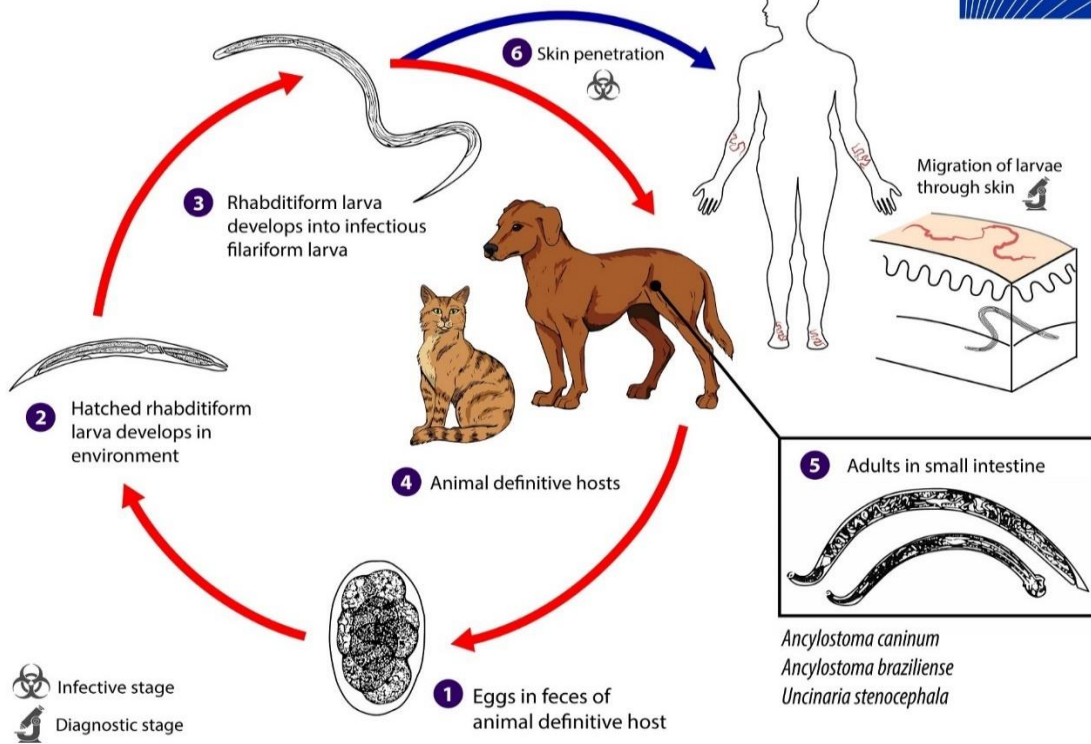


Figure 2. 1: Life cycle of Hookworm (CDC, 2022)

2.5 Pathophysiology of Hookworm Infection

The pathophysiology of hookworm infection entails a complex relationship between the parasite and the host's immune system. Hookworms, namely *Necator americanus*, are parasitic roundworms that feed on blood, leading to tissue injury, inflammation, and blood loss in the host (Shooraj & Mahdavi, 2022b). The infection commences when larvae penetrate the skin, then migrate to the lungs, ascend to the trachea, and ultimately settle in the small intestine, where they nourish themselves by consuming blood, resulting in iron deficiency anaemia (Razavi et al., 2024; Chaparro & Suchdev, 2019).

The immune response to hookworm infection is marked by a Th2-type response, which involves an elevated production of cytokines such as IL-4, IL-5, IL-9, and IL-13, as well as regulatory cytokines like IL-10 and TGF- β (Gaze et al., 2014). This response is linked to increased levels of total and specific IgE, an increase in eosinophils, and the release of histamine by basophils in reaction to hookworm excretory/secretory products and allergens such as calreticulin (Bretscher, 2014). Although a Th2 and regulatory response is present, there is evidence of a Th1 response, as evidenced by the elevation of IFN- γ and IL-2. However, no significant Th17 response is detected during infection β (Gaze et al., 2014). Essentially, the pathophysiology of hookworm infection entails the parasite invading tissues and feeding on blood, resulting in anaemia and deficits in nutrients. The immune response of the host is mainly biased towards Th2, with regulatory components that can regulate the intensity of the infection and potentially affect reactions to other infections or disorders (Muraille et al., 2014). The molecular and immunological components of hookworm pathogenesis is essential in order to create efficient therapies and vaccines (Gaze et al., 2014).

The pathophysiology of hookworm infection in dogs, primarily caused by *Ancylostoma caninum*, is characterised by blood loss, resulting in anaemia and potentially leading to mortality, especially in puppies (Hawdon & Wise, 2021). The parasite's life cycle includes a stage where the worms feed on blood in the intestines, attaching themselves to the mucosa (Howard et al., 2022). Recurrent infections in dogs can trigger immunological responses. However, the details of these reactions and their effectiveness in offering protection are intricate (Razavi et al., 2024).

Surprisingly, even with advancements in deworming and animal care, there is a growing concern about drug resistance in *Ancylostoma caninum*, which could impact the treatment of the disease (Muraille et al., 2014). Additionally, research has demonstrated that vaccinating dogs with irradiation-attenuated larvae effectively stimulates the immune system, resulting in a considerable reduction in worm and faecal egg counts (Versini et al., 2015). This indicates that the dog's immune system can be prepared to react more efficiently to the hookworms when exposed to them again.

2.6 Hematological Changes Induced by Hookworm

Hookworms attach to the intestinal mucosa of their host, feeding on blood and tissue, which leads to various haematological disturbances. Several studies have detailed the mechanisms by which these parasites cause damage, including anticoagulant secretion, which results in blood loss and leads to anemia. The review by De et al., (2016) provides a foundational understanding of these mechanisms, highlighting the parasite's life cycle and its interaction with the host's blood supply. Multiple studies have reported significant haematological changes in dogs infected with hookworms. These changes primarily include anaemia, alterations in red blood cell (RBC) indices, leukocytosis, and eosinophilia (Furtado et al., 2024; AL-Salami et al., 2023; Li et al., 2023; da Silva et al., 2019; Radakovich et al., 2017; De et al., 2016a).

2.6.1 Red Blood Cell (RBC)

One of hookworm infection's most notable haematological effects is a decrease in the RBC count. The blood-sucking of hookworms in the intestinal tract leads to chronic blood loss,

resulting in anemia. This anaemia's severity correlates with the infection's intensity and duration. Chronic infections lead to a progressive decline in RBC count, emphasizing the need for timely diagnosis and treatment (Allison, 2004).

Ancylostoma caninum, a specific type of hookworm, is known to cause blood loss. The parasite's feeding habits consist of breaking down erythrocytes, which results in blood loss and the possibility of the host developing iron deficiency anemia (McCown & Specht, 2011). It has been shown that the production of red blood cells in dogs infected with hookworms may be enhanced at levels considered normal, indicating a compensatory reaction to the blood loss produced by the parasites (Roberts, 2016). Anderson et al. (2013) documented a significant decrease in RBC count values in dogs infected with *Ancylostoma caninum*, finding that the severity of anaemia correlated with the intensity of the infection (Anderson et al., 2020). Li et al., (2023) highlighted morphological changes in red blood cells, such as poikilocytosis and anisocytosis, in dogs infected with *Ancylostoma braziliense*, suggesting that morphological examination of RBCs can provide additional diagnostic information beyond standard hematologic parameters.

A study observed a marked decrease in RBC count, and increased levels of reticulocytes, indicating a regenerative response to anemia (Allison, 2004). Even though there was blood loss and possible anaemia, none of the animals in the study showed severe anaemia. This suggests that the bone marrow partially made up for the loss of red blood cells (De et al., 2016a). Unlike the mild symptoms that can occur in adult dogs, puppies can experience severe pathology from infection, which can result in substantial blood loss, anaemia, and

occasionally death. However, several gaps remain, with a lack of longitudinal studies that track these changes throughout the infection and treatment, and the exact mechanisms by which hookworms induce anemia (Dracz et al., 2014).

2.6.2 Hemoglobin (Hb) Concentration

Hemoglobin is essential for oxygen transport in the blood, and its reduction can lead to clinical signs such as lethargy, weakness, and pale mucous membranes (Santos et al., 2017). Hookworm infections in dogs can lead to significant decreases in haemoglobin levels, with studies showing a correlation between infection intensity and anaemia severity. Hookworm infection in dogs leads to anaemia, as hookworms consume the host's blood (Radakovich et al., 2017). Studies show that dogs infected with *Ancylostoma caninum* larvae have the lowest overall hematocrit and haemoglobin concentrations. Heavy hookworm infections result in iron-deficient anaemia, which is directly related to reduced haemoglobin levels. Infected dogs also show significant increases in IgG, which is often associated with the body's response to infection. Hookworm infection results in blood loss and decreased haemoglobin concentrations (Miglio et al., 2023a).

Hasina et al. (2022) found that haemoglobin levels are a reliable indicator of disease progression in canine hookworm infections. (2023) observed significant decreases in haemoglobin levels in dogs infected with *Uncinaria stenocephala*, with increased reticulocyte levels suggesting a regenerative response. (2011) investigated dogs with mixed hookworm infections and found significant reductions in haemoglobin levels associated with hypochromic anaemia. These studies highlight the importance of

monitoring haemoglobin levels alongside reticulocyte counts to comprehensively understand hematologic alterations in infected dogs.

2.6.3 Packed Cell Volume (PCV) or Hematocrit

Hookworm infection in canines has been linked to decreased Packed Cell Volume (PCV) or hematocrit, indicating anaemia. A decrease in PCV is a dependable predictor of anaemia, offering a distinct measurement of the blood's ability to transport oxygen (McCown & Specht, 2011). A study conducted by Aroch et al. (2005) discovered that dogs infected with gastrointestinal helminths, such as *Ancylostoma caninum*, exhibited decreased packed cell volume (PCV) values compared to dogs that were not infected. Similarly, studies found that dogs infected with haemoparasites, including those with intestinal parasites at the same time, had noticeably decreased PCV values (Akbar et al., 2014). The data indicate that hookworm infection in dogs can cause anaemia, as evidenced by reduced PCV levels. Furtado et al. (2024) offer a comprehensive analysis of the clinical progression of canine hookworm infections, demonstrating that the severity of anaemia fluctuated depending on the number of infective larvae the dogs were exposed to. Dogs that were infected with a moderate number of larvae (600 L3) had the lowest hematocrit and haemoglobin levels. On the other hand, dogs infected with more larvae (800 L3) had fewer adult hookworms and experienced less severe anaemia (AL-Salami et al., 2023). This suggests the presence of a possible non-linear correlation between the degree of infection and the severity of anemia.

2.6.4 Mean Corpuscular Volume (MCV)

The Mean Corpuscular Volume (MCV) is a critical parameter that measures the average size of red blood cells (RBCs) (Allison, 2004). In dogs with hookworm infection, MCV can show significant variability, influenced by factors such as the stage of the infection and the dog's overall nutritional status (Akbar et al., 2014). In the early stages of hookworm infection, the MCV may remain within normal ranges. This is because the body might initially compensate for the blood loss by increasing RBC production without significant changes in cell size. However, this phase is usually transient as the infection progresses. In some cases, dogs may exhibit an increased MCV, indicating macrocytic anaemia (Mundim et al., 2008). This can occur if the bone marrow responds to chronic blood loss by producing larger, immature red blood cells (reticulocytes) more rapidly (Francey et al., 2021). Macrocytic anaemia can also result from concurrent deficiencies in essential nutrients such as vitamin B12 and folate, which are vital for DNA synthesis in RBC production. Additionally, certain medications or liver diseases associated with hookworm infections might contribute to macrocytosis (Roberts, 2016).

Conversely, a decreased MCV, indicative of microcytic anaemia, is often seen in chronic hookworm infections (Allison, 2004). This condition typically results from prolonged blood loss, leading to iron deficiency, crucial for haemoglobin synthesis. Without sufficient iron, the body produces smaller red blood cells, resulting in a lower MCV (De et al., 2016a). This form of anaemia can be particularly severe in cases where the dog has a poor diet or other underlying health issues that exacerbate iron deficiency. In some cases, dogs may exhibit a combination of macrocytic and microcytic cells, reflecting mixed anaemia.

This situation can arise when there are multiple underlying factors, such as iron deficiency coupled with a regenerative response to anaemia or other nutritional deficiencies (Mundim et al., 2008).

2.6.5 Eosinophil Count

An increase in eosinophil count, known as eosinophilia, is a typical haematological response to hookworm infection. A particular kind of white blood cell called an eosinophil is essential to the immune system's reaction to parasite diseases, including those caused by hookworms (Kluthcovsky et al., 2020). These cells contain granules filled with enzymes and toxic proteins that can be released to combat and destroy parasitic organisms. When a dog is infected with hookworms, the body's immune system is activated to fight off the parasites (Petrov et al., 2018). Eosinophils are recruited to the site of infection in the intestinal tract, where they degranulate and release their toxic contents to kill the parasites. This immune response results in an elevated eosinophil count in the peripheral blood (Paltrinieri et al., 2024).

Elevated eosinophil levels can thus serve as a valuable diagnostic indicator of hookworm infection and other parasitic diseases in dogs (Li et al., 2023). However, it is important to note that eosinophilia is not specific to hookworm infection alone; it can also be seen in other parasitic infections, allergic reactions, and certain inflammatory conditions (Aroch et al., 2015). Therefore, while an increased eosinophil count can strongly suggest the presence of a parasitic infection, it should be interpreted in conjunction with other clinical signs and diagnostic tests. Francey et al. (2021) found that dogs infected with *Ancylostoma caninum*

larvae showed an increase in eosinophils, with the highest eosinophil counts seen in dogs infected with 600 L3 larvae. According to Bruno et al. (2022), dogs infected with *A. caninum* and other gastrointestinal helminths had increased levels of eosinophils. These data indicate that the eosinophil level is a reliable indicator of dog hookworm infection. It is worth mentioning that the level of eosinophilia can differ depending on the severity of the infection. AL-Salami et al. (2023) provide evidence that dogs infected with different amounts of larvae exhibit differing levels of eosinophilia, suggesting that the eosinophil response may be directly related to the extent of parasite infestation. Furthermore, Petrov et al. (2018) provide evidence that supports the idea that eosinophil counts increase in response to hookworm infection. They detected a significant increase in blood eosinophilia when individuals were orally exposed to hookworm larvae (Petrov et al., 2018).

2.6.6 Hookworm Infection and Anemia

One cause of human iron-deficiency anemia is hookworm infection. After adhering to the intestinal mucosa and feeding on host tissues, including blood, the parasites—mainly *Ancylostoma duodenale* and *Necator americanus*—cause persistent blood loss and iron deficiency (AL-Salami et al., 2023). This condition has significant health implications, especially in regions with a high prevalence of hookworm infections. The severity of anaemia correlates with the intensity of hookworm infection. Studies indicate that light infections might be asymptomatic or cause mild symptoms, whereas moderate to heavy infections can lead to severe anaemia (Kluthcovsky et al., 2020). This condition manifests through fatigue, weakness, and reduced physical and cognitive performance, impairing daily activities and quality of life. Bamanikar et al. (2014) and Hugo (2011) emphasize that

the chronic blood loss caused by these parasites can lead to significant iron depletion over time.

Diagnosing hookworm-induced anaemia can be challenging due to variable clinical presentations. Some patients may not exhibit typical eosinophilia or have negative stool exams for ova or parasites, complicating diagnosis (Bamanikar et al., 2014; Kuo et al., 2010). Boopathy et al. (2014) report rare cases of stomach infestation by hookworms, highlighting the need for a high index of suspicion in endemic areas. Several studies reported the link between hookworm infection and anaemia. A study by Hotez et al. (2005) found that hookworm infection was directly associated with anaemia among school-aged children in rural Brazil. The pilot study in Kintampo North, Ghana, revealed a high prevalence of hookworm infection, particularly among children and adults with specific risk factors such as poor nutritional status and lack of sanitation facilities (Humphries et al., 2011). Similarly, school-aged children in the same region showed a high prevalence of hookworm infection, with modest cure rates following albendazole treatment, suggesting potential drug resistance (Humphries et al., 2013). Anaemia, a common consequence of hookworm infection due to intestinal blood loss, has a substantial economic impact, reducing worker productivity.

The implications of hookworm-induced anaemia are profound, particularly in developing countries with a high burden of parasitic infections. Anemia not only affects individual health but also has broader socioeconomic impacts (Petrov et al., 2018). It impairs physical and cognitive development in children, reduces productivity in adults, and increases the

risk of maternal and perinatal mortality (Shooraj & Mahdavi, 2022b). Effective control of hookworm infection and its associated anaemia requires a scientific approach. Regular anthelmintic treatments, as recommended by the World Health Organization, are vital in reducing the parasite burden in affected populations (Oliveira-Arbex et al., 2022). Also, iron supplementation and fortification programs can help address the iron deficiency caused by chronic blood loss. Improving sanitation and promoting health education are also essential components of prevention. Educating communities about proper hygiene practices, such as wearing shoes to avoid soil contact and using latrines, can reduce the risk of infection (Oliveira-Arbex et al., 2022). Addressing the public health challenge of hookworm-induced anaemia requires integrated strategies, combining medical treatment with preventive measures to reduce the burden of this debilitating condition.

2.7 Global Epidemiology of Hookworm Infection

Hookworms are parasites that live in the intestines of humans and animals. They enter the body through contact with soil and feed on blood (Loukas et al., 2016b). About 450 million people around the world have long-term hookworm infections, which cause the loss of 2.1 million healthy life years and over \$100 billion in global economic losses each year. These infections are most common in poor areas, affecting 438.9 million people yearly (Clements & Alene, 2022). Regions like Sub-Saharan Africa, the Pacific Islands, India, Southeast Asia, Latin America, and the Caribbean have about 576 million cases (Haldeman et al., 2020).

The main species infecting humans are *Ancylostoma duodenale* and *Necator americanus*. In Southeast Asia and the Pacific, *Ancylostoma ceylanicum* is also found. This species infects humans as well as animals like dogs and cats (Zibaei et al., 2020). *N. americanus* is the most common type, causing 79% of cases, while mixed infections with *Ancylostoma spp.* occur in 5% of cases (Bartsch et al., 2016). The number of worms varies from person to person, with some carrying most of the worms. Hookworm infections are highest in middle-aged or older adults and more common in males, possibly due to their work environments (Zibaei et al., 2020). Factors like genetics, environment, and poor sanitation affect how hookworms spread and how severe the infections are (Hossain & Bhuiyan, 2016b; Ugbomeh et al., 2018). Hookworms also infect dogs worldwide, especially in warm and humid areas with many stray dogs (Traub et al., 2021). For example, 56.6% of dogs in Brazil, 45% in Sri Lanka, and 81.3% of infected dogs in Nigeria had hookworms. Common species include *Ancylostoma caninum* and *A. ceylanicum* (Oliveira-Arbex et al., 2022; Bandaranayaka et al., 2019; Massetti et al., 2021). In Thailand, studies showed *A. caninum* and *A. ceylanicum* in household dogs, which can contaminate the environment (Wongwigkan & Inpankaew, 2020).

Regions like Southeast Asia and Sub-Saharan Africa, with warm climates, poor sanitation, and many stray dogs, have high rates of canine hookworm infections. Zoonotic species like *A. ceylanicum* and *A. braziliense* can spread to humans, posing health risks. Regular monitoring and control measures are needed to prevent human infections (Haldeman et al., 2020; Alhassan et al., 2022).

2.8 Prevalence of Hookworm Infection in Ghana

2.8.1 Human Hookworm Infection

Hookworm infections in Ghana exhibit endemic characteristics with a notably patchy geographical distribution. Various studies have documented the prevalence of hookworm infections, showing this uneven spread (Amissah-Reynolds & Ofori, 2023; Amissah-Reynolds et al., 2016; Boyko et al., 2020). For instance, in northern Togo, which borders Ghana, hookworm infections are endemic across all examined villages (Ziem et al., 2006a). Economic development in Ghana has contributed to a decline in some neglected tropical diseases (NTDs). However, the reduction in hookworm prevalence has been less pronounced. This disparity highlights hookworm infections' persistent public health challenge despite overall economic progress.

The prevalence of hookworm infections varies significantly across different populations within Ghana. Inhabitants of an orphanage showed a prevalence of 1% (Duedu et al., 2015). Among children under five years old in the Oti region, the prevalence was higher at 13.16% (Abaka-Yawson et al., 2020). Pregnant women in Southern Ghana had a lower prevalence of 0.3% (Adu-Gyasi et al., 2018). Patients in a psychiatric hospital demonstrated a hookworm carriage rate of 13.5% (Agmas et al., 2021). a study by Ziem et al. (2006b) reports a significant reduction in hookworm prevalence from 86.9% to 23.4% in northern Ghana following four rounds of albendazole treatment among school children. Yelifari et al. (2005) indicate a 50.6% prevalence of hookworm infections in northeastern Ghana. A study estimates that over 230,000 people are infected with hookworm in northern Togo, which borders northern Ghana, suggesting a high prevalence

in the adjacent regions (Pit et al., 1999). Adu-Gyasi et al. (2018) document a 12.1% prevalence of hookworm in the middle belt of Ghana, while Magalhães et al. (2011) report a 3.2% prevalence of hookworm mono-infection nationwide. Ziem et al. (2004) show a 75.5% prevalence of 'hookworm-like' eggs in stool samples in the Garu area of northern Ghana. Ziem et al. (2006) find an 86.9% prevalence of hookworm in northern Ghana. Kulinkina et al. (2023) discuss reducing hookworm prevalence from 8.6% to 3.1% between 2008 and 2015 after large-scale preventive chemotherapy.

These variations suggest that certain regions and demographic groups are more affected than others, indicating areas where targeted interventions could be particularly beneficial. The distribution and prevalence of hookworm infections in Ghana are influenced by age, gender, and socioeconomic conditions (Agmas et al., 2021). For example, a study in the Rio de Janeiro metropolitan area revealed that socioeconomic status, indicated by the material deprivation index, was associated with the prevalence of intestinal parasites, including hookworm. This finding indicates the importance of effectively addressing socioeconomic disparities to control and reduce hookworm infections. Despite the general trend towards a reduction in hookworm prevalence. Current data suggest that specific populations, such as children and institutionalized individuals, continue to experience high infection rates (Kurumadas et al., 2021; Ziem et al., 2006a). Future research should focus on Identifying the underlying reasons for the persistently high prevalence in specific populations, evaluating the effectiveness of current intervention strategies, and exploring the role of environmental and socioeconomic factors in the transmission of hookworm. Additionally, there is a need for comprehensive, region-specific studies better to

understand the local epidemiology of hookworm infections in Ghana. More effective public health strategies can be developed to combat hookworm infections in Ghana by addressing these gaps.

2.8.2 Canine Hookworm Infection

Studies show that canine hookworms are common in Ghana, though rates vary by region. In the Greater Accra Region, Johnson et al. (2015) found 46.8% of dogs had hookworm infections, showing a need for better control in urban areas where dogs and people interact. In Kintampo North, Boyko et al. (2020) found 43% of dog samples contained hookworm eggs, and 47% tested positive for *Necator americanus* DNA, showing the risk of transmission to humans. Amissah-Reynolds et al. (2024) reported hookworm rates of 64% in Wa, 57% in Techiman, and 44% in Kumasi, with differences linked to environment, dog care, and health programs. Earlier research found lower rates, like 9% in the Ashanti Region reported by Amissah-Reynolds et al. (2016), which may reflect rising infections or different testing methods. Cats also carry hookworms, with 22.2% infected in the Ashanti Region (Amissah-Reynolds & Ofori, 2023). This shows that both dogs and cats spread the parasites, increasing the risk for humans.

Hookworms in dogs are a public health concern, especially with zoonotic species like *N. americanus* possibly spreading to humans (Sahimin et al., 2017). To control infections, it is important to educate the public, deworm pets regularly, improve veterinary care, and keep environments clean. Molecular tests, like those used by Boyko et al. (2020), can help

track infections more accurately. Continued efforts are needed to lower hookworm rates and protect both animals and people.

2.9 Treatment and Control of Hookworm Infections in Dogs

2.10 Anthelmintic Drugs and their Mode of Action

Anthelmintics are the main way to treat parasitic worms like hookworms in dogs because they are cheap and easy to use. Choosing the right drug or combination depends on the need for treatment, how bad the infection is, and if the worms are resistant to the drugs. Keeping these drugs effective is important since there are no better options available. Anthelmintics are grouped into classes based on how they work and their chemical makeup, such as benzimidazoles, macrocyclic lactones, imidazothiazoles, and tetrahydropyrimidines (Zamanian & Chan, 2021). Each class works in its own way to ensure safety and effectiveness.

2.10.1 Benzimidazoles

Benzimidazoles, like fenbendazole, albendazole, and mebendazole, are commonly used anthelmintics in veterinary medicine to treat parasites like hookworms. These drugs work by binding to β -tubulin in the parasite, blocking the formation of microtubules, which are needed for cell structure, transport, and division (Panic et al., 2014). This stops glucose absorption, drains energy, and eventually kills the parasite. Albendazole is effective against a wide range of parasites, making it a popular choice for general treatments (Rodríguez-Gonzalo et al., 2017). It is typically given to dogs at a dose of 25 mg/kg in a single oral treatment.

Fenbendazole is preferred for its safety and success in treating gastrointestinal parasites like hookworms (*Ancylostoma caninum*). It greatly reduces egg counts in feces and clears infections, making it reliable for regular deworming (Abongwa et al., 2017). Mebendazole works similarly by stopping microtubule formation in parasites, disrupting their cell functions. Though used less often, it is effective against hookworms and other intestinal parasites. Its low absorption in the gut allows for higher concentrations where the parasites are located, improving its effectiveness (Shaharyar & Mazumder, 2017). Benzimidazoles target helminths specifically because they bind more strongly to parasite β -tubulin than to the host's. Food can affect how well the drug is absorbed, and maintaining the right drug levels at the parasite's location is key to their success. Studies confirm benzimidazoles are effective in treating hookworm infections in dogs (Keri et al., 2015).

2.10.2 Macrocyclic Lactones

Macrocyclic lactones are widely used in veterinary medicine because they work well against both internal and external parasites. These include drugs like ivermectin, milbemycin oxime, selamectin, and moxidectin (Ballesteros et al., 2018). They work by making parasite nerve and muscle cells more permeable to chloride ions, causing paralysis and death (Bowman et al., 2014).

Ivermectin is effective against many parasites, including hookworms. It targets their nervous system, causing paralysis and killing the parasites (Clarke et al., 2019). Milbemycin oxime is useful for preventing and treating parasitic infections, especially against hookworm larvae. Selamectin, mostly used for external parasites like fleas and

ticks, also works against hookworm larvae by paralyzing their nerve cells (Mahaparale & Banju, 2019). Moxidectin is stronger and lasts longer than other macrocyclic lactones, making it effective for stubborn infections like hookworms (Doherty et al., 2018). Its long half-life keeps the drug active in the body for a longer time, offering extended protection. These drugs can be given orally, on the skin, or by injection. Fatty meals can improve their absorption since they are fat-soluble. They spread widely in the body, especially in fatty tissues and the liver.

Macrocyclic lactones target parasites because their chloride channels are different from those in animals. These channels are more common and important in parasites, making the drugs effective without harming the host (Becskei et al., 2020). The long-lasting effects and wide tissue distribution help fight both larvae and adult parasites (Dyary, 2016).

2.10.3 Imidazothiazoles

Levamisole is a widely used deworming drug that works by targeting parasites' muscle cells. It activates specific receptors (nicotinic acetylcholine receptors), causing continuous muscle contraction. This results in paralysis and removal of the worms (Abongwa et al., 2017). Although newer deworming drugs are available, levamisole is still important in veterinary medicine because it acts quickly and is effective against many worm types (Dyary, 2016). It has been proven to work well against parasites like hookworms and intestinal nematodes. Studies show it reduces worm numbers and clears infections in dogs when used at the right doses and intervals (Hamilton & Rath, 2018).

However, levamisole can have side effects, such as affecting the immune system or causing stomach issues. Proper dosing and monitoring are crucial to avoid these problems. Once inside the body, levamisole works mainly in the digestive system by attaching to receptors in the worm's muscle cells. This causes the parasite's muscles to contract and leads to paralysis, making the worm lose its grip and be expelled from the host (Brishty et al., 2021; Moser et al., 2019).

2.10.4 Tetrahydropyrimidines

Tetrahydropyrimidines are drugs used to treat parasitic worms in animals, including dogs. The main types are pyrantel pamoate, pyrantel tartrate, and morantel (Godhani et al., 2014). These drugs are effective against worms like roundworms and hookworms (Kopp et al., 2007). Pyrantel pamoate is commonly available as chewable tablets, oral suspensions, or paste. It works well for gastrointestinal worms, with the typical dose for dogs being 5 mg/kg given by mouth (Kamat et al., 2023). For severe infections, another dose may be given after 2-3 weeks. Pyrantel is often combined with other drugs to treat more worm types. For example, combining pyrantel pamoate with praziquantel (e.g., Drontal Plus) can treat tapeworms, roundworms, and hookworms. The dosage for this combination depends on the dog's weight, such as ½ tablet for 2-4 kg, 1 tablet for 5-10 kg, and so on (Sepehri et al., 2015).

Morantel tartrate is usually used in livestock but sometimes in dogs, either in feed or as an oral drench. Its dosage for dogs is also around 5 mg/kg, though it is less common than pyrantel. Combining tetrahydropyrimidines with other drugs increases their usefulness by

covering a wide range of parasites (Jadhav et al., 2019). These drugs are very safe, with rare and mild side effects. Pyrantel pamoate is not well absorbed in the gut, so it stays where the worms are and reduces the risk of side effects. This makes it safe for young, pregnant, or sick animals (Bhosale et al., 2021). However, it should be avoided in animals allergic to it. Pyrantel tartrate is better absorbed and can be used in situations needing systemic action (Garibov et al., 2016).

2.11 Current status of Anthelmintic Resistance

Anthelmintic resistance (AR) is increasingly being observed in various helminths across different animal species and drug groups worldwide. Studies in Europe have shown rising resistance to widely used anthelmintics, including benzimidazoles, tetrahydropyrimidines, imidazothiazoles, and macrocyclic lactones (Milović et al., 2022). A survey by Bhattacharyya (2019) found high resistance to benzimidazoles (BZ) and macrocyclic lactones (ML) on goat farms in Poland, though resistance to levamisole (LEV) was relatively low. In Romania, Jeddi et al. (2022) recorded the first cases of gastrointestinal nematode resistance to BZs and MLs in goats. Resistance has also been reported in *Haemonchus contortus* to newer drugs like monepantel and amino-acetonitrile derivatives (Kamat et al., 2023).

Research shows resistance can develop shortly after a drug is introduced, sometimes leading to the closure of sheep and goat farms (Enne et al., 2008). Sheep have been found to develop resistance to imidazothiazoles, tetrahydropyrimidines, and macrocyclic lactones within three to nine years of use (Výrostková et al., 2021; Silva et al., 2020; Enne et al.,

2008). Multidrug resistance is becoming a major concern, with reports of *Haemonchus contortus*, *Teladorsagia*, and *Trichostrongylus* showing resistance to BZs, imidazothiazoles, and MLs across Europe. In tropical regions where parasites are more prevalent, excessive anthelmintic use accelerates resistance, posing significant public health risks (Molla et al., 2006).

Governments often lack awareness or fail to act on the urgency of AR. In Ethiopia, commonly used anthelmintics include benzimidazoles (e.g., albendazole, triclabendazole), macrocyclic lactones (e.g., ivermectin), imidazothiazoles (e.g., tetramisole, levamisole), tetrahydropyrimidines (e.g., pyrantel pamoate), and salicylanilides (e.g., niclosamide) (Dyary, 2016). Misuse and overuse of these drugs have led to resistance in various worms across regions. Wondimu and Bayu (2022) reported resistance in *Trichostrongylus*, *Teladorsagia*, and *Haemonchus* spp. in goats from Haramaya, Ethiopia, to drugs like albendazole, tetraclozan, ivermectin, and tetramisole. Similarly, Mphahlele et al. (2021) documented significant AR in gastrointestinal nematodes infecting sheep in Limpopo Province, South Africa.

2.12 Resistance Mechanism of Anthelmintic

The main cause of resistance to benzimidazoles is changes in the β -tubulin protein. One well-known change is the transformation of phenylalanine-200 to tyrosine (F200Y) in β -tubulin (Appiah-Twum et al., 2023). Other important mutations include the replacement of phenylalanine with tyrosine at position 167 (F167Y) and the change from glutamic acid to

alanine at position 198 (E198A). These genetic changes stop benzimidazole from binding properly to tubulin, leading to resistance (Kamat et al., 2023).

Resistance to macrocyclic lactones, such as ivermectin, is mainly due to the widespread use of these drugs (Paras et al., 2018a). These drugs target chloride channels in parasites, and resistance can occur due to mutations in the genes that produce these channels. Studies have shown that mutations in the GluClR gene are linked to resistance to ivermectin in *Haemonchus contortus* (Atif et al., 2019). Another factor in resistance is P-glycoproteins (Pgps), which remove drugs from the parasite's cells, lowering the drug's effectiveness. PGP-2 is particularly associated with resistance to macrocyclic lactones (Luo et al., 2017). Also, enzymes that break down drugs, like CYP34/35, are found in higher levels in drug-resistant *H. contortus* strains. Resistance to drugs like levamisole and pyrantel is linked to changes in nicotinic acetylcholine receptors (nAChRs), specifically the L-type receptors (Lynagh & Lynch, 2012). These drugs normally activate the receptors, causing paralysis in the parasites. Resistance occurs when the genes that produce these receptors are less active, as seen in *H. contortus* and *Ancylostoma caninum*. Shortened versions of receptor subunits, like *acr-8b* and *unc-63b*, are also linked to resistance in *H. contortus*, *Trichostrongylus colubriformis*, and *Trichostrongylus circumcincta* (Tuersong et al., 2022).

2.13 Causes of Anthelmintic Resistance

Recent anthelmintics have a success rate of about 99% when targeting vulnerable parasites. However, some parasites with resistance or tolerance to the treatment survive. These

resistant parasites spread through the environment and contaminate pastures, leading to the birth of more resistant parasites due to selective pressure. The development of anthelmintic resistance (AR) is influenced by several factors, with the frequency of treatments being the most important (Kotze et al., 2020). How often anthelmintics are used is a key factor in how quickly resistance develops. Regular treatments speed up resistance because the surviving parasites have a reproductive advantage over the susceptible ones for about two to three weeks after treatment (Fissiha & Kinde, 2021a). This reproductive advantage allows the resistant parasites to become the dominant strain quickly. Large-scale preventive treatments have been shown to promote resistance in helminths. However, resistance can be prevented by treating about 80% of the flock instead of the entire population (Doyle & Cotton, 2019). Strategic treatment plans, like avoiding treatments during high transmission periods and targeting high-risk groups in the flock, can reduce resistance (Gasbarre, 2014).

Improper use of anthelmintics is a major cause of resistance. For example, using ocular weight assessments to determine doses is often inaccurate, leading to underdosing (Kotze & Prichard, 2016). Underdosing allows worms with one copy of the resistance gene to survive, which helps resistant strains develop. Accurate weight measurements and following the correct dosage are crucial to prevent resistance (Hodgkinson et al., 2019).

Some parasites are already resistant to certain treatments before exposure. Anthelmintic resistance (AR) exists because resistance genes are present in the population before anthelmintic use (Ahuir-Baraja et al., 2021). These resistance genes remain rare without treatment, as they disadvantage the worms carrying them. However, using an anthelmintic gives resistant worms a survival advantage, allowing them to reproduce faster than

susceptible worms (Sangster et al., 2018). This leads to an increase in resistant genes, and eventually, resistance becomes established. In cases where resistance is recessive, only worms with two copies of the resistance gene can survive the treatment, while worms with one copy of the gene will die. Genetic factors like gene flow and genetic diversity in parasite populations can affect how quickly resistance spreads (Geerts & Gryseels, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

Chapter Three of this study explores the detailed technique and geographical background of research carried out in the Bono East Region of Ghana. The chapter provides a complete description of the research area and study design. The study focuses on the selection criteria for animals in eight districts. The study's methodology also includes the strategies used for sampling, the criteria for selecting dogs, and the specific procedures for collecting data and analyzing it in the laboratory. Ethical considerations and respect for animal welfare norms demonstrate the study's commitment to acceptable research techniques.

3.2 Study Area

The study took place in the Bono East Region, located in central Ghana. This region was formed in 2019 when the Brong-Ahafo Region was divided. It is bordered by the Savannah Region to the north, Bono Region to the west, Ashanti Region to the south, and Volta Lake to the east. The area has a climate with mostly favorable conditions throughout the year (GHPC, 2021). The main vegetation consists of forests and fertile soil. The dry season usually lasts from December to April, while the wet season occurs from July to November, with average rainfall ranging from 750 to 1050 mm per year. Temperatures are highest at the end of the dry season, and lowest during December and January (GHPC, 2021). The dry, hot period between December and early February is marked by the Harmattan winds from the Sahara, causing temperatures to vary between 14°C (59°F) at night and 40°C

(104°F) during the day. The region is known for agricultural activities, including livestock farming, and has urban centers like Techiman, Nkoranza, Kintampo, and Atebubu. The study was carried out in eight of the eleven districts in Bono East, excluding Pru West, Pru East, and Sene West (Figure 3.1).

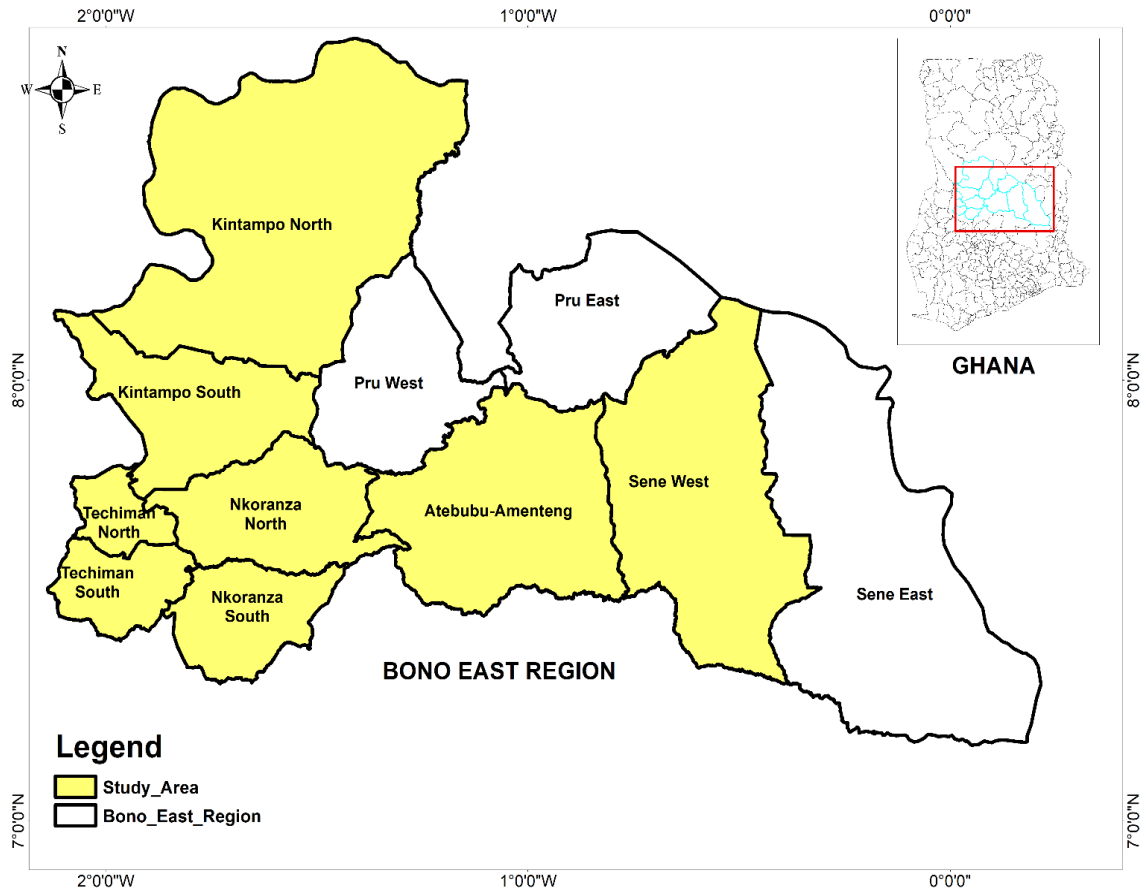


Figure 3.1: Map of Bono East indicating the various study District (Source: Design using ArGIS 10.7)

3.3 Population and Sample Size

The study focused on domestic dogs in the Bono East Region of Ghana. A previous study on hookworms in the area indicated a prevalence rate of 47%, which was used to calculate the sample size (Amisshah-Reynolds et al., 2024). Using this prevalence, a desired precision

of 5%, and a 95% confidence level, the sample size was calculated to be 301 dogs. The formula used for the calculation was $n = (z^2 \times P \times (1-P)) / d^2$, where n is the required sample size, Z is the Z-value (1.96 for a 95% confidence level), P is the estimated prevalence (0.47), and d is the desired precision (0.05). Based on this calculation, the final sample size was set to include a minimum of 301 dogs.

3.4 Experimental Design and Animals Selection

The study employed an Experimental control trial between March and August 2024. Sampling was done in veterinary facilities, farms, and households in both urban and rural areas to capture the diversity of the district's socio-economic and environmental factors. Dogs from various districts in the region with or without a history of anthelmintic usage and known hookworm infections were selected for the study. Hookworm-infected dogs were recruited from each district and grouped into cohorts. Each cohort consisted of three treatments (Albendazole (ABZ), Pyrantel (PP), and Niclosamide (NIC) units, as well as two control groups (Positive and Negative control). Two groups of 76 dogs each received Albendazole or Pyrantel, with 70 dogs receiving Niclosamide. For the control groups, 27 dogs were given a placebo to serve as the negative control, and an additional 40 healthy dogs were included as positive controls. Dogs were recruited in collaboration with local veterinary authorities across the region. Priority was given to households with a history of anti-helminthic usage for their pets, and animals were recruited based on the presence of hookworm infections detected through initial faecal examinations.

3.5 Sampling Technique

The study used a combination of stratified and random sampling to select the animals. First, stratified sampling was applied to divide each district into rural and urban areas, ensuring all regions were represented. Then, random sampling was used to select animals from each area to account for differences in hookworm prevalence and drug effectiveness across the region. Dogs that met the inclusion criteria were randomly chosen for the experimental control trial. Each dog was tagged with a unique identification number, and their health was checked at the start of the study and during the 14-day follow-up visits. Fresh fecal samples were collected from each dog every four days.

3.6 Inclusion and Exclusion Criteria

Dogs were included in the study if their owners were available and agreed to have them screened. The study included puppies (0-1 year), young dogs (1-2 years), and adults (2 years and older) to see if age affected how well the drug worked. Dogs of different breeds and purposes were included to check for any breed-specific differences. Only dogs with confirmed hookworm infections were part of the trial. However, pregnant dogs, very young puppies, and dogs with serious health problems that could affect the study were excluded to ensure accurate results. Dogs that had been dewormed in the last three months were also excluded.

3.7 Anthelmintic Treatment of Dogs

Dogs were divided into four groups and orally treated with suspensions of Albendazole, a Benzimidazole derivative (Batch No. F230320, Hebei New Century Pharmaceutical

Industry, Shijiazhuang, China), Prazivet plus® (Praziquantel-50mg/Pyrantel pamoate-144mg/ Febantel-150mg), Batch No. P2-230704, Smith & Kenner Pharmaceuticals, India), Niclosamide, (Batch No. NSC-178296, Smith & Kenner Pharmaceuticals, India) or a placebo (control group). ABZ and NIC were administered at a dose of 50mg/kg of the dog's body weight. In contrast, Pyrantel was administered at 34.4mg/kg of body weight (i.e. manufacturer's recommended doses for dogs). The efficacy of drugs was evaluated using both in vivo (the faecal egg count reduction test (FECRT)) and in vitro methods. Egg counts were recorded in each treatment unit before drug administration (Day 0) and on Days 4, 8 and 14 after treatment. The post-treatment egg screening followed the same procedure as the pre-treatment. All dogs in the control group received a placebo on day 0. In adherence to ethical considerations, they were treated at the end of the study. At the beginning of the study, the weight of the dogs was recorded, and the dosage of the anthelmintic drug was adjusted based on the instructions provided by the manufacturers.

3.8 Fecal Collection

To collect faeces from dogs, a gloved finger was lubricated with water-based gel. The finger was gently inserted into the rectum, massaged a bit, and then removed. About 5-6 grams of faeces were collected and placed in labelled containers, which were kept on ice. For puppies, a small lubricated spatula was used to collect faeces from the anus while the puppy was held still. About 3-6 grams of faeces were scooped and put into labelled containers, also kept on ice. If rectal collection wasn't possible, fresh stool samples were taken right after the dog defecated, using gloved hands to collect the top part of the stool. These samples were placed in labelled containers and sent to the lab.

3.9 Blood Sample Collection

Blood samples were taken from each dog at two times: Day 0 and Day 14. The dogs were gently held to reduce stress, and the area for the blood draw, usually the cephalic vein, was cleaned with antiseptic. About 5 ml of blood was collected using a clean needle and syringe. Two samples were taken from each dog: one for blood analysis, placed in a tube with EDTA (an anticoagulant), and the other for chemical analysis, placed in a tube without anticoagulant to get serum. The EDTA tube was gently shaken to mix the blood and anticoagulant. All samples were collected in the morning and sent to the Animal Health Clinical Laboratory in Kintampo, Bono East Region.

3.10 Laboratory Analysis

3.10.1 In vivo - Fecal Egg Count Using McMaster

Fecal egg counts (FECs) were done three times using the McMaster Technique. The method described by Paras et al. (2018) was followed to process the samples and count the eggs. First, 3g of feces was measured and put into a container. Then, 28 ml of NaCl flotation solution was added, mixed, and left to soak for 5 minutes. After soaking, the mixture was stirred and strained into another container. Both chambers of the McMaster slide were filled with the mixture using a pipette. The slide was examined under a microscope with 10× and 40× lenses to find and count hookworm eggs by their shape and size. All eggs within the slide's grid were counted with the 10× lens, including those on the grid lines if more than half the eggs were inside the grid. The total count from both chambers was multiplied by 50 to get the eggs per gram (epg). The effectiveness of treatments was measured by the percentage reduction in fecal egg count, calculated using

the formula: $FECR (\%) = ((\text{Average Egg count pre-treatment}) - (\text{Average Egg count post-treatment})) / (\text{Average Egg count pre-treatment}) \times 100$. If the reduction in egg count was less than 95% or the lower 95% confidence limit was under 90%, resistance was considered present (Coles et al., 2006).

3.10.2 In vitro- Egg Hatch Assay

Faecal samples were taken from the dogs' rectums and treated using a 1.2 g/ml sodium chloride solution. About 100-200 eggs were placed in each well of a 24-well plate. For the PP and NIC stock solutions, 50mg of each drug was dissolved in 100 ml of a 50% dimethyl sulfoxide (DMSO) solution. The ABZ suspension was made by mixing 10% DMSO and distilled water in a 40:60 ratio. Serial dilution with distilled water was done to get five concentrations of 0.025, 0.05, 0.065, 0.08, and 1.0 $\mu\text{g/mL}$ in 1% DMSO for each drug. A liquid-based method was used in 24-well plates, following a protocol with some changes (Kurumadas et al., 2021). The final DMSO concentration was 1% in each well for both treatment and control wells, which is safe for parasites (Kurumadas et al., 2021). Each treatment concentration was repeated three times. The last two wells had control eggs that were incubated without treatment, while the other wells had increasing drug concentrations. The wells were kept at 42-48°C for 48 hours, and the number of larvae was counted using a microscope. The egg hatch inhibition (EHI) and IC50 values for each drug were calculated. The EHI was determined using this formula:

$EHI (\%) = ((\text{Eggs hatched in the control group}) - (\text{Eggs hatched after drug treatment})) / (\text{Eggs hatched in the control group}) \times 100$.

3.11 Hematological and Biochemical Test

Hematological analysis was performed using blood samples collected with an anticoagulant (EDTA). These samples were then analyzed using Hematology Analyzers (Sysmex XN-1000, Model Number: XN-1000, manufacturing in Japan), Hematological parameters, including haemoglobin, packed cell volume (PCV), total leukocyte count (TLC), differential leukocyte count (DLC), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Biochemical analysis focused on specific serum parameters, including total protein, albumin, globulin, creatinine, ALT and AST. The Beckman Coulter AU480 Clinical Chemistry Analyzer (USA) was employed for this study. This model is known for its high throughput, reliability, and precision in biochemical analysis. The blood samples without anticoagulants were allowed to clot, and the serum was separated by centrifugation. The obtained serum samples were stored in appropriately labelled cryovials and maintained at a temperature of -20°C to preserve their integrity before biochemical analysis. The serum samples were analyzed using Clinical Chemistry Analyzers, employing specific assays to measure the biochemical parameters accurately.

3.12 Data Analysis

Descriptive statistics were used to find the prevalence of hookworm infections and the egg counts (epg) before and after treatment for each group. Logistic regression analysis was used to look at the link between hookworm infection and factors like sex, age, purpose, and settlement. Paired t-tests compared the pre-treatment and post-treatment egg counts

within the same group, while independent t-tests compared treated and untreated groups. The effective half-inhibitory concentration (IC₅₀) values from the in vitro study were calculated using a logistic model fitting curve (Dose-Response (Inhibition)). All data analysis was done using R statistical software 4.4.1 and GraphPad Prism version 10.1.

3.13 Ethical Consideration

Before the study began, ethical approval was obtained from the Animal Ethics Committee at KNUST (KNUST/AREC/C.1, 0063). Dog owners gave their informed consent. Animal welfare rules were followed carefully during sample collection and treatment. The effectiveness tests were conducted based on guidelines from the World Association for the Advancement of Veterinary Parasitology (WAAVP).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the study's findings on hookworm infections and anthelmintic efficacy among dogs in the Bono East Region, Ghana. The research objectives outlined in Chapter One guided the study, which included determining the prevalence of hookworm infections, identifying risk factors associated with infection, assessing anthelmintic efficacy and investigating the impact of infections on health parameters. The following discussion delves into the implications of these findings and their significance in addressing the challenges of hookworm infections in the region.

4.2 Hookworm Prevalence

Of 491 surveyed dogs, 266 (54.2%) tested positive for hookworm infection ($X^2 = 6.517$, $p = 0.010$). Age was strongly associated with infection rates ($X^2 = 26.136$, $p < 0.001$), with puppies exhibiting the highest prevalence (69.8%), followed by young dogs (55.2%) and adult dogs (41%). The purpose for which dogs were kept significantly influenced infection rates ($X^2 = 12.288$, $p = 0.0021$), with hunting (64.1%) and those used for security (55.6%) or companion dogs (44.6%) showing varying prevalence rates. Settlement type also played a role, with rural areas experiencing a significantly higher prevalence (84.2%) compared to urban areas (35.2%) (**Figure 4.1**). Eight (8) out of eleven (11) districts examined show varying prevalence ranging from 45% to 74% based on geographical area (**Figure 4.2**).

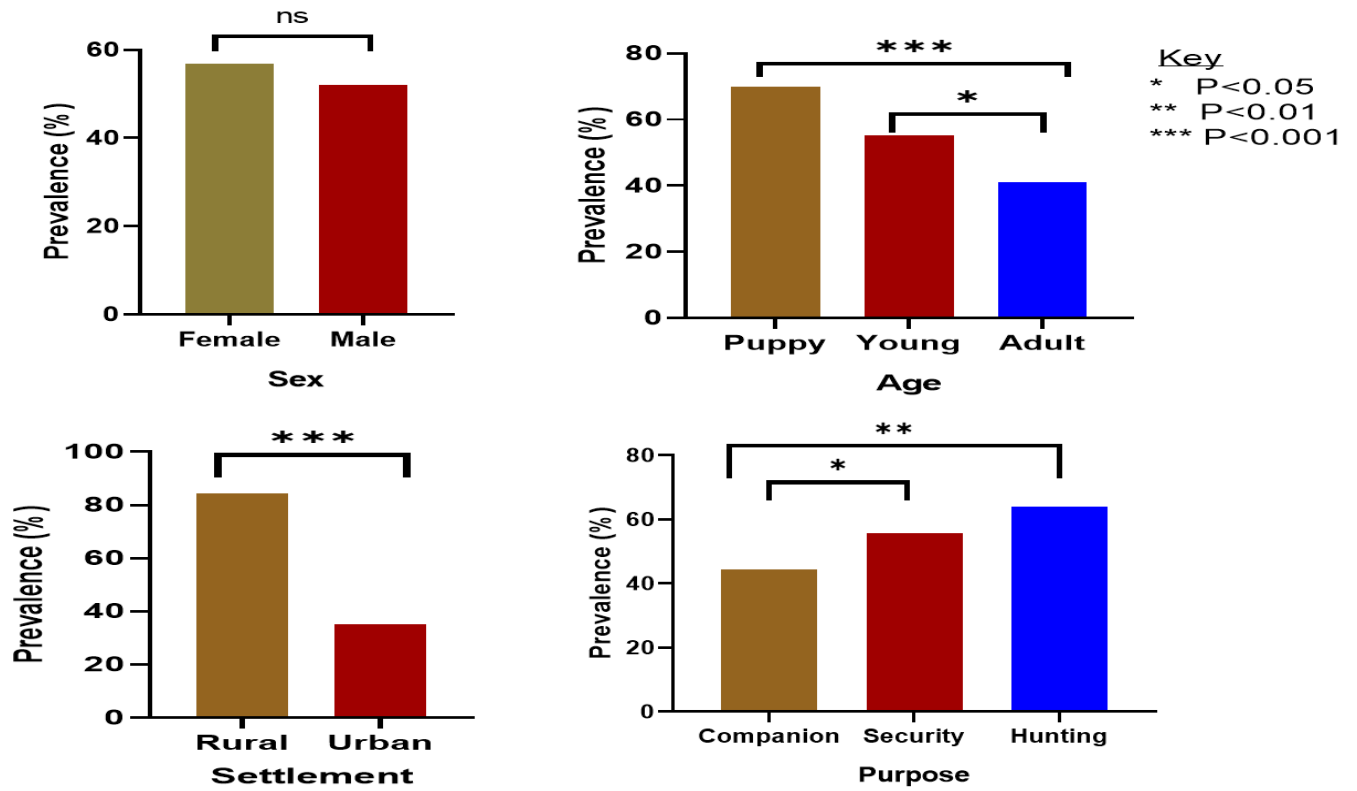


Figure 4.1: Hookworm Prevalence among Dogs in Bono East

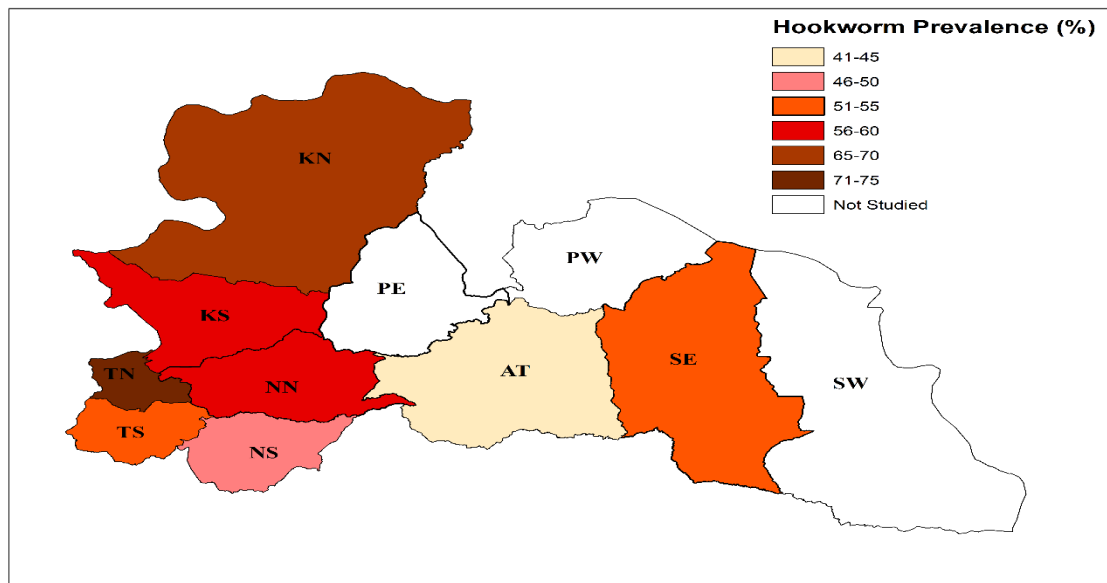


Figure 4.2: District Prevalence of Hookworm infection in Dogs

4.3 Risk Factor Analysis of Hookworm Infection among Dogs

The analysis of risk factors revealed several important associations (Table 4.1). Puppies were much more likely to be infected than adult dogs (AOR = 3.625, 95% CI = 0.534–4.421, $p = 0.003$). However, there was no significant difference in infection rates between young dogs and adults (AOR = 2.127, 95% CI = 0.759–2.539, $p = 0.451$). When considering the dogs' purpose, those used for hunting had significantly higher chances of hookworm infection compared to companion dogs (AOR = 1.56, 95% CI = 0.337–1.932, $p = 0.026$). No significant link was found for dogs kept for security purposes (AOR = 0.786, 95% CI = 0.478–1.292, $p = 0.342$). The type of settlement also played a role in infection risk, with dogs in rural areas showing a slightly higher chance of infection than those in urban areas (AOR = 1.519, 95% CI = 0.994–2.321, $p = 0.05$).

Table 4.1: Risk factor of hookworm infection among dogs in Bono East Region

Predictor	Adjusted Odds Ratio (AOR)	95% C.I. AOR	p-value
Age			
Adult	Reference		
Young	2.127	0.759, 2.539	0.451
Puppies	3.625	0.534, 4.421	0.003
Purpose			
Companion	Reference		
Security	0.786	0.478, 1.292	0.342
Hunting	1.56	0.337, 1.932	0.026
Settlement			
Urban	Reference		
Rural	1.519	0.994, 2.321	0.05

4.4 Efficacy of Anthelmintics on Hookworm Infection

The study evaluated the efficacy of three anthelmintics, Albendazole (ABZ), Pyrantel (PP), and Niclosamide (NIC), in reducing helminth egg counts in infected dogs (**Figure 4.3**). Albendazole had a negative efficacy of -69%, as seen by a considerable increase in post-treatment egg counts (**Table 4.2**). Pyrantel showed a low efficacy of 2.5%, but Niclosamide exhibited a high efficacy of 95%. In vitro, inhibition percentages of Albendazole, Pyrantel and Niclosamide were assessed at various concentrations (100 µg/mL, 80µg/mL, 65 µg/mL, 50 µg/mL, and 25 µg/mL). Pyrantel showed the highest inhibition at 100 µg/mL, followed by niclosamide at 96.73% (**Figures 4.5 & 4.6**). Albendazole showed lower inhibition at 86.49%. Niclosamide consistently demonstrated higher inhibition compared to pyrantel and albendazole at lower concentrations. At 65 µg/mL, niclosamide showed the highest inhibition (78.16%), followed by pyrantel (72.3%) and albendazole (50.71%). The lowest IC₅₀ value for niclosamide was 29.19 µg/ml, with pyrantel at 38.20 µg/ml and Albendazole at 52.41 µg/ml. Higher R-squared values indicated a stronger relationship between drug concentration (**Table 4. 3**).

Table 4 2: In Vivo Efficacy of Anthelmintic in Reducing Helminth Egg Counts in Dogs

Anthelmintic	Location	No. of Dogs	Egg Count Before Treatment (Days Before/Day 0)	Pre-Treatment	Egg Count After Treatment (Days After Day 0)	Post-Treatment	Efficacy (%)
ABZ	AT	11	0	642±67.3	4,8,14	876±112.1	-36
	KN	10	-2,0	685±93.0	4,8,14	1265±190.6	-85
	KS	6	-2,0	490±59.5	4,8,14	569±57.4	-16
	NN	11	-1,0	784±195.7	4,8,14	930±113	-19
	NS	8	-1,0	1580±66.1	4,8,14	1098±161.6	31
	SW	10	0	575±82.1	4,8,14	613±119.6	-7
	TN	10	-1,0	679±74.2	4,8,14	1163±220.6	-71
	TS	10	-1,0	2286±127.1	4,8,14	3098±605.5	-36
	Total	76	-2-0	730±46.7	4-14	1229±125.8	-69
PP	AT	9	0	963±84.3	4,8,14	1679±355.6	-74
	KN	10	-2,0	632±64.2	4,8,14	1288±194.4	-104
	KS	6	-2,0	446±47.3	4,8,14	518±44.1	-16
	NN	9	-1,0	1054±136.6	4,8,14	372±89.7	65
	NS	10	-1,0	2247±454.5	4,14	1015±186.5	55
	SW	9	0	744±201.6	4,8,14	106±34.5	86
	TN	13	-1,0	567±114.2	4,8,14	1248±346.5	-120
	TS	10	-1,0	908±79.1	4,8,14	917±217.8	-1
	Total	76	-2-0	957±91.9	4-14	934±100	2.5
NIC	AT	12	0	1808±178.5	4,8,14	65±11.7	96
	KN	10	-2,0	1460±196.5	4,8,14	115±22.2	92
	KS	10	-2,0	2808±112.1	4,8,14	85±14.6	96
	NN	7	-1,0	1494±203.4	4,8,14	148±17.7	90
	NS	10	-1,0	1390±210.3	4,8,14	48±11.7	96
	SW	10	0	1192±136.1	4,8,14	76±27.7	94
	TN	11	-1,0	762±167.5	4,8,14	42±7.4	94
	Total	70	-2-0	1559±68.2	4-14	82±18.5	95

AT-Atebubu, KN-Kintampo North, KS-Kintampo South, NN- Nkoranza North, SW-Sene West, TN-Techiman North, TS- Techiman South, ABZ-Abendazole, PP-Prazivet Plus®, NIC-Niclosamide

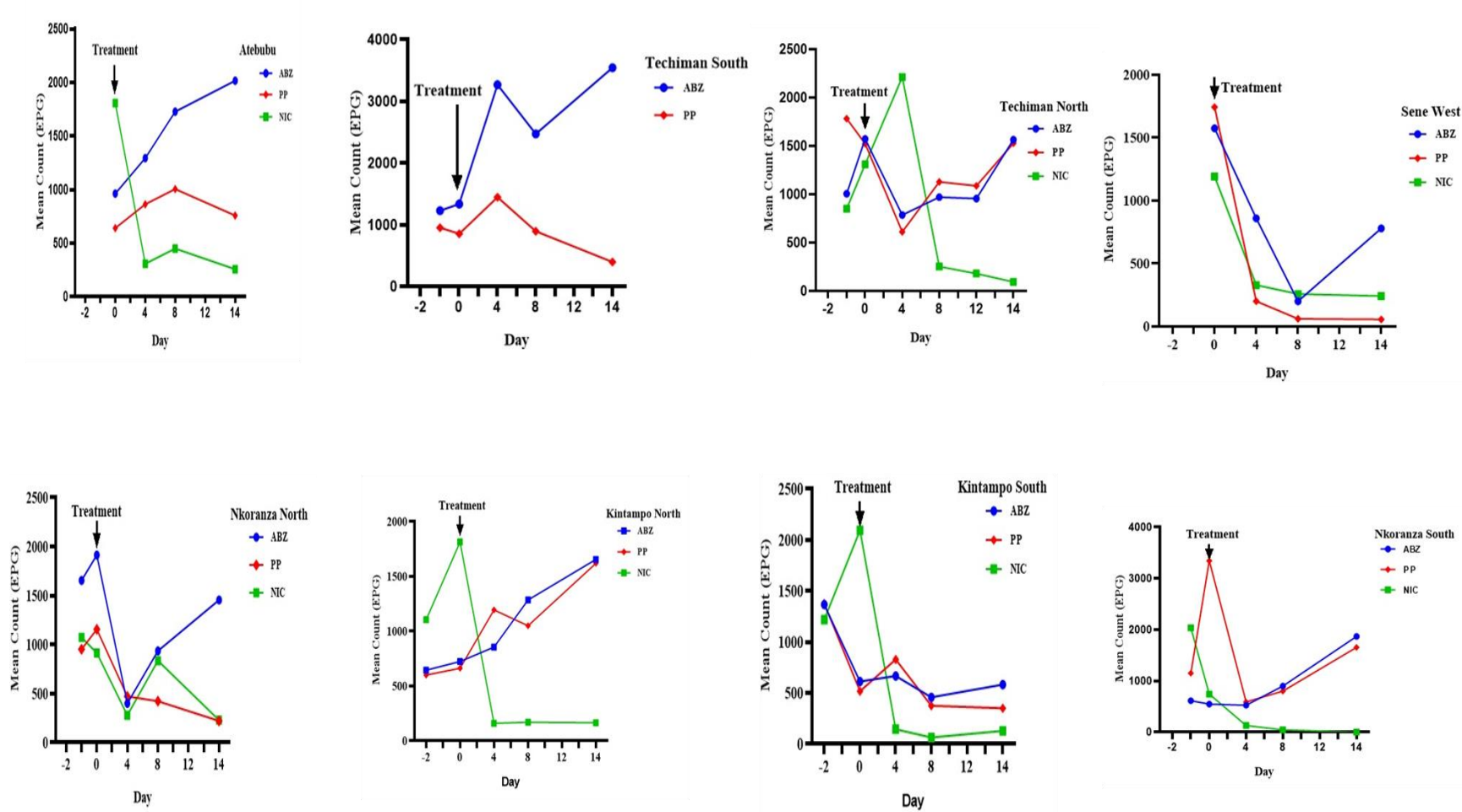


Figure 4.3: Fecal egg counts taken before and after treatment of dogs with ABZ-Albendazole, PP- pyrantel and NIC-Niclosamide within the Eight Districts. (Districts names are shown on each panel. Arrows indicate the day of treatment (Day 0))

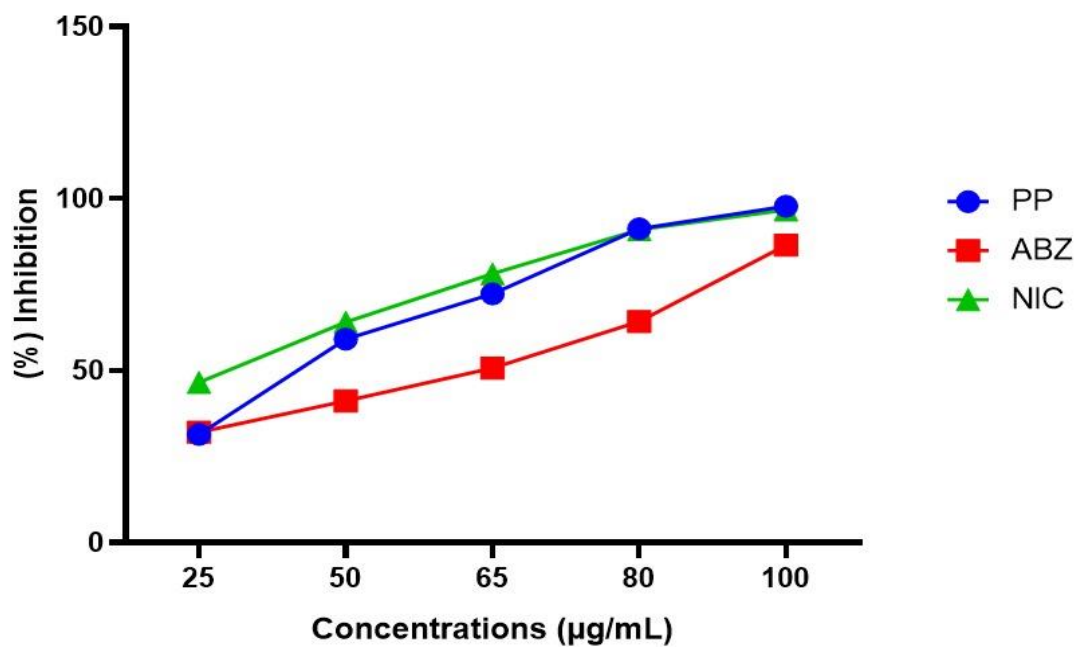


Figure 4.4: Mean plot of Anthelmintic inhibition against different concentration

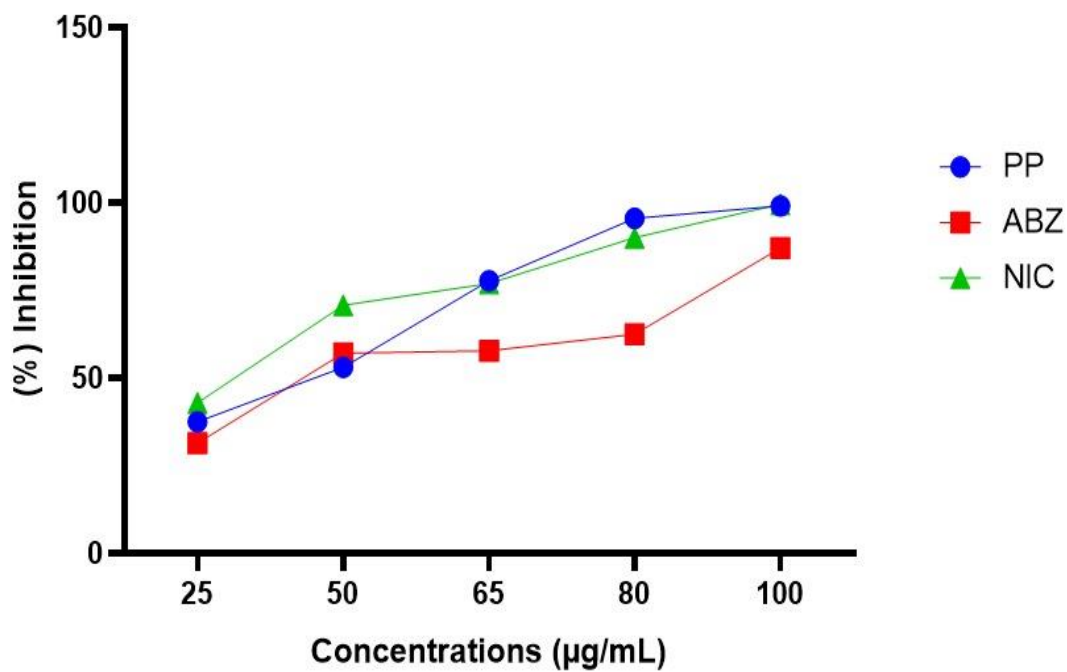


Figure 4.5: Median plot of Anthelmintic inhibition against different concentration

Table 4 3: Comparative IC_{50} Values of Anthelmintic for Treating Hookworm Infections

Anthelmintic	IC_{50}	R Squared	95% CL
Abendazole	52.41	0.7306	29.79 - 72.37
Pyrantel	38.2	0.9870	27.29 - 48.62
Niclosamide	29.19	0.9941	15.30 - 39.52

IC_{50} - Half Maximal Inhibitory Concentration.

4.5 Haemato-biochemical Parameters of Dogs

Dogs that were heavily infected had considerably reduced number of red blood cells (RBC) with an average count of 4.07 ± 0.27 , compared to dogs with light infections (average count of 5.63 ± 0.43) and moderate infections (average count of 5.60 ± 0.44) ($p = 0.025$) (Table 4). Infection severity also had an impact on haemoglobin (Hb) levels and mean corpuscular volume (MCV) (**Figure 4.6**). The percentage of eosinophils increased in correlation with the severity of the infection ($p = 0.003$). There were notable alterations in the biochemical markers, with total protein, albumin, and globulin levels fluctuating with the severity of infection. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels were, however, within reference ranges (**Figure 4.6**).

The study assessed the impact of anthelmintics on haematological and biochemical parameters in dogs over 14 days. Haematological findings showed significant increases in red blood cell (RBC) counts, haemoglobin (Hb) levels, and packed cell volume (PCV) across all treatment groups, with NIC treatment resulting in the highest RBC increase, reaching 6.30×10^6 . NIC also improved mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration

(MCHC). Biochemically, total protein levels rose significantly in the PP (31.64 ± 2.79 to 56.07 ± 2.82 g/L) and NIC (44.02 ± 1.87 to 63.35 ± 1.29 g/L). Globulin levels decreased significantly in both PP (62.99 ± 6.29 to 37.03 ± 1.72 g/L) and NIC (56.29 ± 3.29 to 39.11 ± 1.88 g/L) groups (**Table 4.4**).

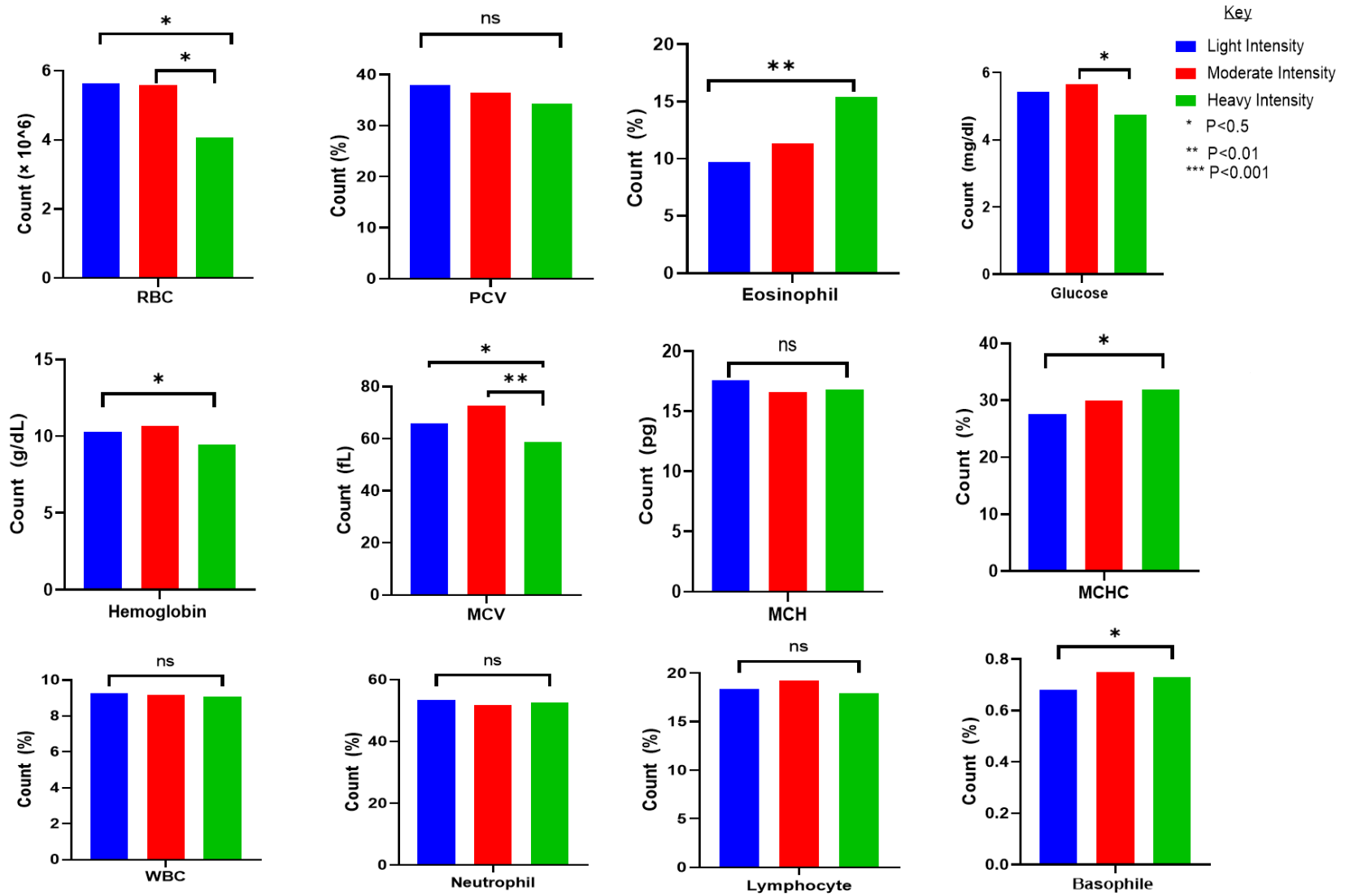


Figure 4.6: Hematological changes Parameters in Canine with Varying Degrees of Infection, Light intensity is <500 EPG, Moderate intensity range between 500-2000 EPG, High intensity is >2000, ns: Not statistically significant

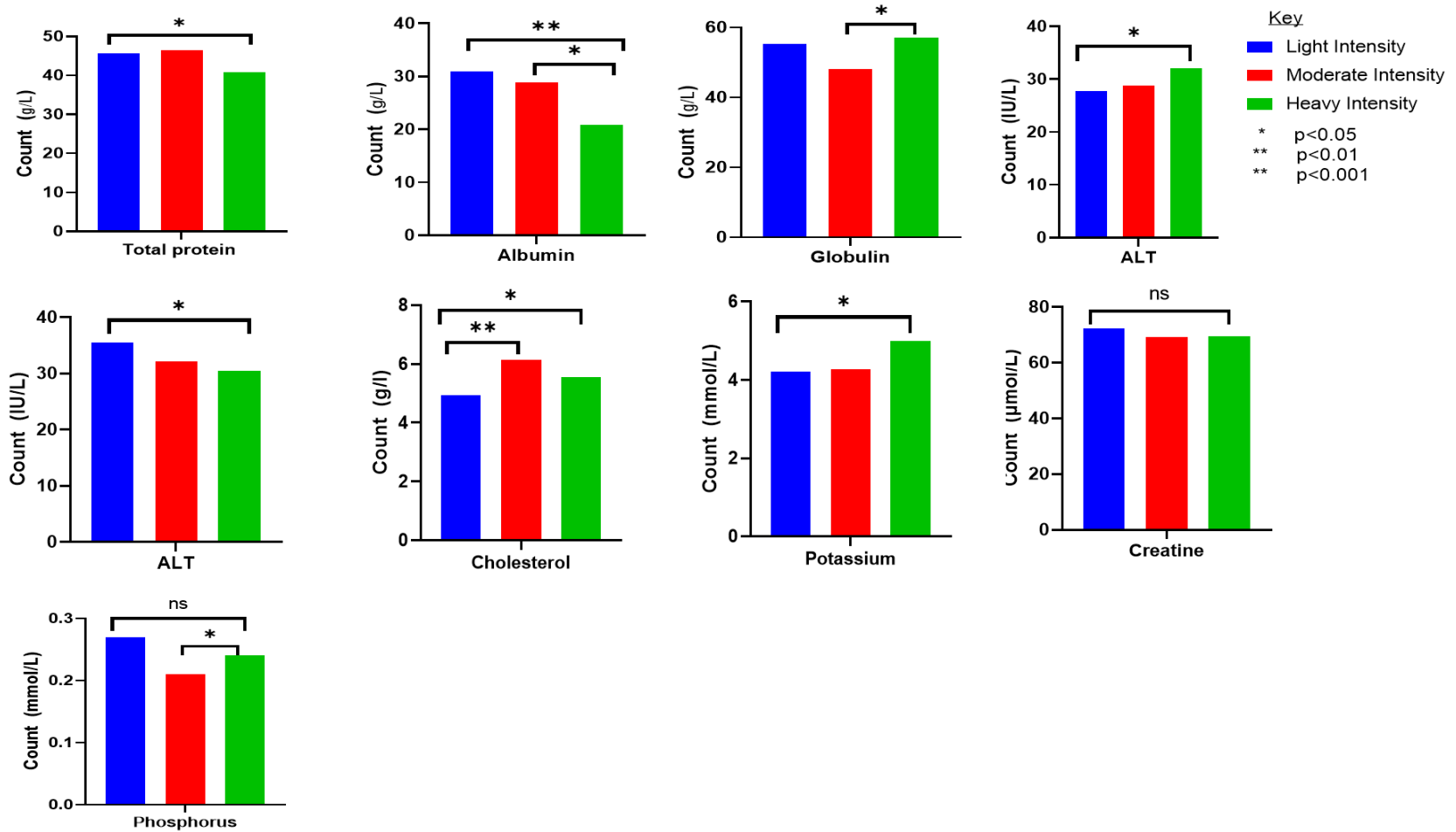


Figure 4.7: Biochemical changes of Parameters in Canine with Varying Degrees of Infection, Light intensity is <500 EPG, Moderate intensity range between 500-2000 EPG, High intensity is >2000, ns: Not statistically significant

Table 4.4: Haemato-biochemical parameters of different treatment group on day 0 and 14

Parameters	Reference point	ABZ		PP		NIC		Placebo		Healthy Control	
		Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
RBC ($\times 10^6$)	5.5-8.5	5.36 \pm 0.48	6.14 \pm 0.18	5.16 \pm 0.46	6.44 \pm 0.33	5.08 \pm 0.030	6.30 \pm 0.24	5.21 \pm 0.70	4.89 \pm 0.55	6.90 \pm 0.53	6.06 \pm 0.51
Hb (g/dl)	12-18	8.44 \pm 0.50	12.25\pm0.68*	10.21 \pm 1.02	13.11\pm1.37*	7.42 \pm 0.40	14.76\pm0.86*	10.61 \pm 1.07	9.46 \pm 0.97	11.87 \pm 1.35	12.56 \pm 1.06
PCV (%)	37-55	35.29 \pm 1.51	38.70 \pm 1.53	33.51 \pm 3.13	38.49 \pm 2.54	31.49 \pm 2.3.	43.99\pm1.69*	32.61 \pm 3.04	35.45 \pm 2.53	39.86 \pm 0.99	42.67 \pm 3.18
MCV (fL)	60-77	58.69 \pm 3.23	60.50 \pm 3.07	65.01 \pm 4.52	58.86 \pm 4.71	59.42 \pm 4.52	50.99 \pm 5.45	71.15 \pm 1.76	71.88 \pm 1.84	77.31 \pm 3.83	59.46 \pm 5.42
MCH (pg)	19-24	18.34 \pm 1.24	20.86 \pm 1.45	20.39 \pm 1.04	25.59 \pm 2.06	16.28 \pm 1.95	17.19 \pm 2.04	23.34 \pm 1.55	24.55 \pm 1.71	11.84 \pm 1.23	12.97 \pm 1.45
MCHC (%)	30-36	29.64 \pm 1.15	32.07 \pm 1.25	31.29 \pm 2.16	36.63 \pm 1.91	33.16 \pm 1.29	34.55 \pm 1.54	34.09 \pm 0.84	35.74 \pm 1.62	27.88 \pm 2.44	28.48 \pm 4.07
WBC ($\times 10^3$)	6.0-17.0	10.34 \pm 0.46	12.42 \pm 0.64	7.11 \pm 0.46	12.31 \pm 0.80	12.29 \pm 1.01	13.81 \pm 0.92	11.41 \pm 0.65	12.96 \pm 0.88	8.13 \pm 1.07	8.60 \pm 1.21
Neutrophil (%)	60-70	44.56 \pm 3.17	47.09 \pm 2.64	56.62 \pm 3.06	58.17 \pm 2.88	39.62 \pm 2.28	44.96 \pm 3.14	63.02 \pm 4.66	63.44 \pm 4.06	49.13 \pm 5.17	48.45 \pm 4.55
Monocyte (%)	3-10	3.72 \pm 0.28	6.68 \pm 0.30	6.87 \pm 0.63	8.34 \pm 0.75	6.36 \pm 0.60	8.36 \pm 0.44	6.09 \pm 0.66	7.55 \pm 0.43	9.39 \pm 0.75	10.53 \pm 0.86
Lymphocyte (%)	12-30	26.94 \pm 3.16	28.34 \pm 3.16	14.11 \pm 0.66	15.51 \pm 0.66	17.91 \pm 1.07	19.31 \pm 1.07	19.42 \pm 2.62	20.82 \pm 2.62	20.65 \pm 4.56	22.05 \pm 4.56
Eosinophil (%)	2-10	10.54 \pm 0.77	11.04 \pm 0.77	10.03 \pm 1.00	11.53 \pm 1.00	9.91 \pm 0.61	16.41\pm0.61*	8.52 \pm 0.96	10.02 \pm 0.96	10.40 \pm 1.16	11.90 \pm 1.16
Basophil (%)	0-1	0.49 \pm 0.05	1.19 \pm 0.05	0.86 \pm 0.17	1.56 \pm 0.17	0.99 \pm 0.21	1.69 \pm 0.21	0.72 \pm 0.06	1.42 \pm 0.06	0.71 \pm 0.05	1.41 \pm 0.05
Biochemical											
Total protein (g/L)	56-71	55.09 \pm 4.92	59.79 \pm 3.75	31.64 \pm 2.79	56.07\pm2.82*	44.02 \pm 1.87	63.35\pm1.29*	33.20 \pm 2.41	31.16 \pm 2.92	57.18 \pm 3.28	58.20 \pm 3.70
Albumin (g/L)	30-36	27.70 \pm 1.02	35.13 \pm 0.60	27.60 \pm 1.13	36.43 \pm 2.46	26.61 \pm 0.49	37.77\pm0.88*	25.44 \pm 1.49	28.54 \pm 0.13	33.52 \pm 0.75	35.92 \pm 1.25
Globulin (g/L)	25-38	56.12 \pm 4.56	36.35\pm0.92*	62.99 \pm 6.29	37.03\pm1.72*	56.29 \pm 3.29	39.11\pm1.88*	52.56 \pm 5.79	50.16 \pm 2.23	34.32 \pm 0.57	30.76 \pm 2.50
Glucose (mg/dl)	4.0-6.3	5.45 \pm 1.17	5.85 \pm 0.27	4.77 \pm 0.39	6.77 \pm 0.40	6.06 \pm 0.45	7.13 \pm 0.36	6.50 \pm 0.20	6.96 \pm 0.30	5.74 \pm 0.23	4.98 \pm 0.32
Cholesterol (mmol/l)	3.87-8.39	5.95 \pm 0.25	5.87 \pm 0.27	4.77 \pm 0.42	6.14 \pm 0.52	4.33 \pm 0.31	4.97 \pm 0.37	6.54 \pm 1.03	5.52 \pm 0.35	6.22 \pm 0.40	7.84 \pm 0.15
ALT (IU/L)	13-69	30.35 \pm 3.30	31.22 \pm 3.08	43.73 \pm 4.18	45.60 \pm 4.08	37.06 \pm 3.91	38.40 \pm 4.58	33.76 \pm 6.48	27.42 \pm 5.81	14.98 \pm 0.54	23.34 \pm 5.74
AST (IU/L)	18-55	26.50 \pm 2.73	32.78 \pm 2.69	23.49 \pm 3.32	27.73\pm3.44*	39.20 \pm 3.54	47.37\pm3.29*	28.46 \pm 3.07	35.06 \pm 3.61	34.16 \pm 5.00	34.88 \pm 4.51
Creatinine (μ mol/L)	54-122	72.75 \pm 5.84	90.20\pm8.48*	66.97 \pm 5.58	68.00 \pm 3.58	84.73 \pm 3.30	84.17 \pm 3.80	76.18 \pm 4.58	72.52 \pm 4.59	45.52 \pm 8.36	51.08 \pm 3.69
Phosphorus (mmol/L)	0.84-1.83	0.25 \pm 0.03	1.45 \pm 0.04	0.30 \pm 0.05	1.60 \pm 0.06	0.21 \pm 0.03	1.41 \pm 0.07	0.16 \pm 0.03	1.28 \pm 0.07	0.26 \pm 0.05	0.98 \pm 0.19
Potassium (mmol/l)	3.9-5.3	4.13 \pm 0.52	4.82 \pm 0.16	4.60 \pm 0.55	5.47 \pm 0.47	6.03 \pm 0.70	5.67 \pm 0.36	2.96 \pm 0.50	4.48 \pm 0.35	4.52 \pm 0.45	4.38 \pm 0.42

4.6 Discussion

The study found a prevalence of 54.2% among the 491 dogs surveyed. This finding highlights a significant health concern in the region. The high prevalence rate reported in this study is consistent with previous studies in Ghana (Amissah-Reynolds et al., 2024; Boyko et al., 2020; Amissah-Reynolds et al., 2016; Johnson et al., 2015b) and other countries (Illiano et al., 2023a; Dao-Hua et al., 2020; Hossain & Bhuiyan, 2016b; Mahdy et al., 2012). Hookworm prevalence rates vary across regions, with significantly higher rates in Africa due to various factors such as warm climates, poor sanitation, and limited animal healthcare. Lower prevalence of hookworms reported in Europe (Illiano et al., 2023b; Genchi et al., 2021; Umbrello et al., 2021) is likely due to better access to veterinary care and stricter public health measures.

The worldwide extent and persistent nature of the challenge of hookworm infections in dogs necessitates periodic surveillance. In Africa, microscopic examination is the most common method for identifying hookworm infections. However, microscopy has low sensitivity and may potentially lead to underreporting or misidentification of less common or emerging species. The few molecular-based studies in Africa have identified *Necator americanus* (Boyko et al., 2020), *Ancylostoma caninum* (Mulinge et al., 2019), *Ancylostoma braziliense* (Kamani et al., 2021), *Uncinaria stenocephala* (Shchelkanov et al., 2021), and *Ancylostoma ceylanicum* (Merino-Tejedor et al., 2019) as the species infecting dogs. Although *Ancylostoma ceylanicum* was originally thought to be restricted to Asia and the Pacific regions, it has been reported in Tanzania and Africa. This finding necessitates further molecular surveillance in dogs to elucidate the distribution of

hookworm species within the sub-region. Understanding the risk factors for infection is essential for creating active control programs. Factors such as age, purpose, location, housing styles and deworming status can increase the likelihood of dogs becoming infected with parasites (Zendejas-Heredia et al., 2022; Amissah-Reynolds et al., 2024). In the present study, puppies exhibited higher infection rates. This is due primarily to the lack of acquired immunity and behaviours that increase exposure to contaminated environments. Hunting dogs also showed a high prevalence of hookworm infections, which concurs with Amissah-Reynolds et al. (2024), who reported a 72.1% infection rate in hunting dogs. This can be attributed to their frequent exposure to outdoor environments favouring hookworm transmission. Rural areas reported significantly higher hookworm prevalence (84.2%) than urban areas (35.2%). Amissah-Reynolds et al. 2016 also reported a higher prevalence of the parasites in poor settlement sites. Studies from Ghana, Holland, and Slovakia corroborate the trend of higher parasitic infections in rural settings (Dudlová et al., 2016; Kyei et al., 2015; Nijse et al., 2016). Higher rural prevalence may be due to more stray dogs contaminating the soil with parasite-infested excrement. This highlights the need for better sanitation and veterinary care in these areas.

Significant changes in blood and biochemical parameters were observed in dogs with moderate and heavy infections. Previous studies show a decrease in red blood cell (RBC) count and haemoglobin levels, especially in dogs with heavy infections (Kebbi et al., 2020; Kim et al., 2020; Wyszmołek et al., 2020). These changes are due to the blood loss caused by the parasites, which attach to the intestinal wall and feed on the host's blood, leading to anaemia (Miglio et al., 2023b). Eosinophilia in heavily infected dogs is a typical immune

response to parasites, as seen in studies from Kenya and Nigeria (Omobowale et al., 2017; Kitaa, 2014). The lower mean corpuscular volume (MCV) in heavily infected dogs suggests microcytic anaemia caused by iron deficiency from chronic blood loss (Salem et al., 2015). This finding suggests the importance of early treatment to avert further complications. Hypoalbuminemia and hypoproteinemia in heavily infected dogs were concordant with hookworm-infected dogs' reports (Audu, 2024; Craven & Washabau, 2019; Pierini et al., 2019). These conditions are likely due to protein loss through the intestines and reduced production by the liver, as the infection stresses the body. Changes in liver enzymes, such as the decrease in ALT and increase in AST, suggest that the liver may be under strain or damaged. This observation aligns with the findings from necropsy studies in Egyptian dogs reported by Abbas et al., (2023) in their systematic review. Investigating detailed eosinophil responses and other immune markers is essential. This can enhance treatment plans and contribute to developing effective vaccines since vaccines are not available for hookworm treatment.

The high prevalence of hookworm infection indicates the need to evaluate the efficacy of available anthelmintics in Ghana. This trial is the first randomized control study comparing the efficacy of Albendazole, Pyrantel and Niclosamide in treating hookworm infections in dogs in Ghana. Egg counts increased after Albendazole, in all 72 dogs, resulting in a total efficacy of -64%. This increase may be due to density-dependent fecundity, where worms susceptible to albendazole are eliminated, thus allowing female hookworms to produce more eggs (Kopp et al., 2007; Serafim et al., 2014). This suggests possible resistance of hookworms to albendazole. This raises concerns about drug resistance, poor drug quality,

reinfection, or biological differences among dog populations in the study area. Similarly, albendazole resistance has been reported in dogs (Paliy et al., 2021; Paredes et al., 2018; Shakoor et al., 2024), although high efficacy of albendazole has been reported in ruminants (Adediran & Uwalaka, 2015; Brahma et al., 2015; Afonso et al., 2014). Previous studies in Ghana have isolated *Necator americanus*, a hookworm known to infect humans in dogs (Boyko et al., 2020). The low efficacy observed in dogs and humans (Humphries et al., 2013) in the study area could be linked to the transmission of zoonotic species. Genetic indicators linked to benzimidazole resistance have been identified in hookworm samples from Ghana, indicating that the use of drugs may enhance the presence of mutations associated with resistance (Orr et al., 2019). This raises concerns about species-specific efficacy tests, calling for treatments tailored to the particular species involved. Low albendazole efficacy could also be attributed to higher average egg count before treatment since lighter infections tend to respond better to benzimidazoles like albendazole than heavier infections (Strydom et al., 2023).

Pyrantel is a widely used anthelmintic for treating hookworm in dogs in Ghana (Johnson et al., 2015). However, widespread reports of pyrantel resistance in canine hookworm in the USA (Castro et al., 2021, 2020; Jimenez Castro et al., 2019; Kitchen et al., 2019) Australia (Dale et al., 2024), and Canada (Nezami et al., 2023) is of concern. The limited efficacy observed could be due to the emergence of drug-resistant strains of hookworms and issues related to underdosage or overdose. Prazivet® (the pyrantel-based drug) used in this study is a broad-spectrum anthelmintic that contains pyrantel pamoate, praziquantel, and febantel. The primary activity against hookworms comes from pyrantel pamoate, with

praziquantel and febantel effective against cestodes (Chai, 2013) and whipworms, respectively (Starkey & Blagburn, 2021). The low efficacy observed with Prazivet® in vivo indicates reduced susceptibility of hookworms to pyrantel (Castro et al., 2021). The higher pyrantel efficacy observed in the in vitro assay could be attributed to suboptimal drug absorption or rapid metabolism (Kitchen et al., 2019). These factors emphasize the necessity of integrating both in vitro and in vivo data when assessing the effectiveness of anthelmintics.

Niclosamide showed higher efficacy in the current study compared to albendazole and pyrantel. While the high efficacy of niclosamide against cestodes and *Toxoplasma gondii* has been reported in pets (Colomb et al., 2023), there is limited information on the efficacy of this drug against hookworms. The mechanism of action of niclosamide involves disrupting the parasite's metabolic processes, making it very effective as compared to other anthelmintics (Colomb et al., 2023). The rare use of niclosamide in the area may influence the development of resistance in this local population. These results suggest that niclosamide could be a strong alternative for treating hookworm infections, especially where traditional treatments have failed. Further research and clinical trials are needed to confirm these findings. This will help determine whether niclosamide should be included in routine treatment plans for canine hookworm infections.

The efficacy of niclosamide and pyrantel in treating hookworm infections was demonstrated by significant improvements in haemoglobin levels, nutritional status, and overall metabolic health. These findings are consistent with studies by Jarry & Alfatlawi,

2023, Kryvoruchenko, 2022 and Silalahi et al., 2022, De et al., 2016 who also reported marked haematological recovery following the use of effective anthelmintics. The strong immune response and reduced inflammation observed in the niclosamide and pyrantel groups further indicate their success in combating hookworm-induced anaemia. In contrast, Albendazole treatment groups showed only moderate improvements, with limited gains in blood and protein levels and a slight reduction in inflammation. This aligns with previous research, which noted that Albendazole has variable efficacy depending on the severity of the infection and the parasite's resistance levels (Santos et al., 2017).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Overall prevalence of dogs were 54.2% (266), with a higher prevalence in puppies (69.8%) and dogs kept for hunting purposes (64.1%). Rural areas had significantly higher infection rates (84.2%) than urban areas (35.2%). Puppies, Hunting, and rural dogs were identified as risk factors for infection. Niclosamide (95%) proved highly efficacious in reducing hookworm infection. The low efficacy of Pyrantel (2.5%) and Albendazole (-69%) suggests antihelmintics resistance. Niclosamide consistently demonstrated superior in vitro inhibition across all tested concentrations compared to pyrantel and albendazole. Hookworm-infected dogs exhibited anaemia, eosinophilia, hypoalbuminemia, and hypoproteinemia. Improvements in haematological and biochemical parameters post-treatment highlight the health benefits of effective anthelmintic intervention.

5.2 Recommendation

Based on the findings of the study, the following recommendations were made;

1. Given its high efficacy in both in vivo and in vitro studies, niclosamide is recommended for use in case of treatment failure with current anthelmintics, especially in resource-limited settings, due to its lower cost compared to other anthelmintics.
2. Further studies are recommended to investigate the possible synergistic impacts of combining niclosamide with other anthelmintics, such as pyrantel and albendazole,

aiming to improve overall effectiveness and minimize the emergence of drug resistance.

3. Extended studies are needed to observe the continued efficacy of niclosamide, pyrantel, and albendazole and the possible emergence of resistance. Consistent surveillance will ensure the continuous efficacy of these drugs in practical use.
4. It is recommended that the efficacy of niclosamide, pyrantel, and albendazole against a broader range of parasitic species be evaluated to understand their spectrum of activity and identify potential limitations or advantages in different contexts.
5. Thorough safety and toxicity assessments are recommended for each anthelmintic, especially at higher concentrations when efficacy is optimized. This will ensure that the suggested dosages are not only efficacious but also safe for prolonged use in diverse animal populations.
6. It is also recommended that the efficacy of Albendazole, Pyrantel, and Niclosamide be conducted in different Regions of Ghana to capture the overall efficacy of these drugs in Ghana.

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