UNIVERSITY OF GHANA

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DEPARTMENT OF ANIMAL BIOLOGY AND CONSERVATION SCIENCE

MACROPARASITES OF SMALL TERRESTRIAL MAMMALS IN GRASSLAND HABITAT OF THE MUNI-POMADZE RAMSAR SITE AND THE UNIVERSITY OF GHANA LEGON MAIN CAMPUS

BY

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DECLARATION

I hereby declare that except for references to other people's work, which I duly acknowledged, this exercise is a result of my own study and this project neither in whole nor in part, has been presented for another degree elsewhere.

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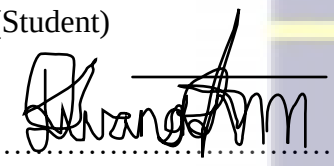
(Student)



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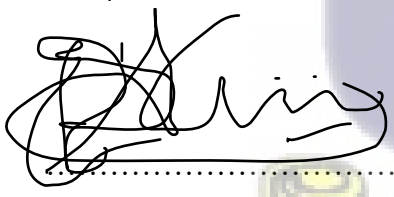

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Dr Benjamin Yeboah Ofori Date:

(Supervisor)

DEDICATION

This work is dedicated to Dr. Bethel Kwansa-Bentum

I am forever indebted to you.

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"My heart has no desire to stay where doubts arise and fears dismay; though some may dwell where these abound, my prayer, my aim is higher ground" (SDAH-625).

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LIST OF ABBREVIATIONS

DALYs-Disability Adjusted Life Years

DNA-Deoxyribonucleic Acid

EID-Emerging infectious disease

ELISA – Enzyme-linked Immunosorbent assay

LASV-Lassa virus

PVA-Polyvinyl alcohol

SAF-Sodium acetate formalin

SDAH- Seventh Day Adventist Hymnal

UG-University of Ghana

WHO -World Health Organisation

TBPs - Tick-borne pathogens

TBEV -Tick-borne encephalitis virus

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ABSTRACT

Zoonosis pose a high risk to public health. It currently accounts for about 61% of global infectious diseases in the world and about 71% of all zoonoses originates from wild animals. Small mammals have a wide geographic distribution and are known reservoirs of zoonotic pathogens. Some small mammal species such as *Mastomys natalensis* are anthropophilic and hence come into contact with humans. This behavior raises genuine concern for potential spillover of zoonotic pathogens from small mammals to humans, domestic animals and livestock. Yet, there is scant information on the ecto- and edo-parasite of small mammals and their zoonotic potential in tropical Savanna ecosystems. To bridge this knowledge gap, I assessed the ectoparasites and gastrointestinal helminths of small mammals in the Savanna ecosystem of the Muni-Pomadze ramsar site, and grassland in the University of Ghana, Legon main campus. The small mammals were captured along line transects using Sherman Live-traps baited with a mixture of corn meal and peanut butter. Captured individuals were examined for mites, lice, ticks, fleas and gastrointestinal helminths. The parasitological matrices: prevalence (%), mean intensity and mean abundance were calculated. The differences of the parasitological matrices between female and male individual were examined using Chi-Square test at a 5% level of significance. A total of 108 small mammal individuals of two species were captured from the two study sites. *Mastomys natalensis* was the dominant species with 95.4% of the total captures (103 out of 108) and *Lemniscomys striatus* made up the rest. In Muni-Pomadze, one ectoparasite (*Haemaphysalis leachi*) and nine gastrointestinal helminths were identified with *Ascaris Hymenolepis* and being the most prevalent (17.6%) and least (2.9%) prevalent helminths. In the grassland in the University of Ghana, Legon main campus, three gastro-intestinal helminths were identified, with the most prevalent being *Ascaris* (32.4%) and *Trichuris* (9.5%) the least). There was no significant difference of helminth infection between sex of mammals in Muni-Pomadze except for

Trichuris [χ^2 =5.0135, df=1, p=0.0251] where infection was significantly higher in females; *Dicrocoelium* [χ^2 =4.1628, df=1, p=0.0413]; and *Hymenolepis* [χ^2 =4.2236, df=1, p=0.0399] where infection was significantly higher in males. In the grassland in the University of Ghana, *Strongyloides* was significantly higher in females [χ^2 =16.1023, df=1, p<0.0001]. In Muni-Pomadze, the mean intensity was higher in females: *Ascaris* (14.33), *Trichuris* (8.33), while in University of Ghana main campus, it was higher in males: *Ascaris* (3.46), *Strongyloides* (7.22) and *Trichuris* (16.5). In the University of Ghana main campus, the mean abundance was higher in females: *Ascaris* (17.91), *Strongyloides* (16.41), *Trichuris* (11.56). Overall, my study has added to the literature on macroparasites in small mammals. I recommend further studies including molecular work to better understand the public health risk posed by these small mammals' intestinal helminths.

Key words: helminth, parasite, Muni-Pomadze, parasite, *Mastomys natalensis*, *Lemniscomys striatus*

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Zoonosis pose a high risk to public health. It currently accounts for about 61% of global infectious diseases in the world and about 71% of all zoonoses originates from wild animals. (Cutler *et al.*, 2010; WHO, 2012). Diseases that are zoonotic appear to be on the rise worldwide (Cantas and Suer, 2014), and about 25% of the world's population have been affected by at least one zoonotic disease (Gilian *et al.*, 2006). Many human infections are zoonotic and for most of these, the source of infection is a wildlife reservoir (Davis *et al*., 2005). Wildlife including mammals are responsible for a large number of diseases which include some of the most virulent diseases like Ebola, rabies and anthrax, and the situation is enhanced by consuming products that are gotten from game (Pavlin *et al*., 2009). The consumption of wildlife animals, and the spillover of infectious diseases from wildlife to food/production animals, should not be overlooked.

Children of school going age are at a higher risk of helminth infection as 25-33.3% of entire world population of at least two helminthic infections (De Silva *et al*., 2003). Since cross-species transmission is the main characteristic of zoonoses, an ecological understanding involving all related hosts is of particular importance for understanding the occurrence of helminth infection in humans (Keeling, 2008). The rate of human infection depends on the prevalence in the animal reservoir, the rate of human-animal contacts and the probability of infection per contact. The frequency, duration and quality of the contact are different in zoonoses transmitted by wildlife, domestic animals or pets (Lloyd-Smith *et al*., 2009).

In several third-world countries, game forms reservoir for foodborne pathogens (Wielinga and Schlundt, 2014). While there is a large body of knowledge of intra-species transmission

of infectious diseases, we know surprisingly little about the dynamics of between-species transmission of zoonotic diseases (Lloyd-Smith *et al*., 2009). The main drivers for infectious diseases outbreaks and emerging infectious diseases remain human population density and growth and their associated antropogenic land-use change (Daszak *et al*., 2001; Patz *et al*., 2004; Jones *et al*., 2008).

Small mammals are one of the highly successful and diverse groups of mammals and found in a wide variety of habitats and play important role in the maintenance of the ecosystem and particularly the food chain. Small mammals act as vital components in various ecosystems either as a prey or predator and sometimes as a carrier/reservoir of diseases (Ezeudu *et al*., 2017). They are also sources of transmission for various viral, rickettsial and bacterial pathogens that cause diseases in humans (Woodhouse *et al*., 2001). When the small mammal population in an area increases, it can be directly related to a corresponding increase in zoonotic diseases in human population (Kataranovski *et al*., 2011). The reason being that they are well recognized to harbour a number of ectoparasites and endoparasites that can cause health problems in humans who live very close to small mammals' populations and even higher risks to those who eat them (Mohd Zain *et al*., 2012).

Zoonotic diseases can be characterized by their route of transmission: (i) direct animal-human transmission; (ii) vector-borne transmission; and (iii) environment (water, soil, food)-borne transmission. Zoonoses can be distinguished in three main levels, according to their transmissibility in humans: (i) diseases like brucellosis and rabies which are transmitted to human without human-to-human transmission, where $R_0>1$ in animals and <1 in humans; (ii) pathogens that spill over into populations with limited human-to-human transmission (eg. Monkey-pox) with R_0 in humans close to 1 and may lead to 'stuttering transmission'; and (iii) diseases like influenza that persist in animal reservoirs but once transmitted to humans may cause persistent and even epidemic transmission in humans with $R_0 > 1$ (Lloyd-Smith *et al*., 2009).

1.2 Justification of the study

New diseases are emerging and are highly infective and also spreads quickly, with the reemergence of some infectious diseases, and these infections have been linked to mammals (Mackenzie *et al*., 2014). About 25% of the world's population have been affected by at least one zoonotic disease (Gilian *et al.*, 2006). The interface between humans, animals, and the environments could be a source of diseases impacting public health and the social and economic well-being of the world population (WHO, 2020). Moreover, multimammate rats are reservoirs and vectors of human pathogens (Lecompte *et al*. 2006, Meheretu *et al*., 2013), including plague and Lassa fever (Green *et al*. 1978, Fichet-Calvet *et al*., 2007). The latter is caused by arenaviruses, some of which have recently been found to be specific to particular rodent species and even intraspecific clades (Gryseels *et al*., 2017, Goüy de Bellocq *et al*., 2020). Similarly, the spread of several viral diseases such as Lassa fever, Ebola virus disease and Coronavirus disease 2019, have been linked to small mammals. The small mammal, *Mastomys natalensis* is a very common and rodents that is found all over Africa (Coetzee, 2010).

Many studies have reported on intestinal parasites in small mammals (Munjita *et al*., 2022). Small mammals are highly prolific and often associated with human dwellings and as a result, either as hosts or reservoirs for some parasite infection (Durnez *et al*., 2008, 2010; Fyfe *et al*., 2010). Small mammals could adapt easily and therefore it would be expected to find difference in its behaviuor (Fyfe *et al*., 2010). Throughout its range a common factor is found: when one compares the population density in the open bush with that surround human habitations, the latter is normally higher and its anthropophilic nature means it could transmit diseases (Fyfe *et al*., 2010).

As an international reserve site, the Muni-Pomadze Ramsar site serves a lot of purposes for both water birds and the human population in the neighbourhood. Not only does it control

flooding but the area also serves as the site where the two *Asafo* companies (warrior groups) in Winneba go to catch live bushbuck (*Tragelaphus scriptus*) for the performance of rituals associated with the annual *Aboakyir* (literally, animal catching) festival. The traditional laws that prohibit the cutting of wood from the sacred forest either for fuel or for any other use are often flouted (Gordon *et al*., 2000). The grassland in the University of Ghana, Legon main campus also serves as a site for academic exercise, and boast of several species of animals including birds. This creates a situation where there appears to be no barriers between the small mammal population and the humans. Several biodiversity studies on wildlife and insects have been carried out on the Muni-Pomadze Ramsar site, and likewise the grassland in the University of Ghana, Legon main campus. Not much is known about the macroparasites that small mammals in the Muni-Pomadze ramsar site and the grassland in the University of Ghana main campus harbour and the potential spill over to the human population that may come into contact with them.

Therefore, this study sought to investigate the ecto- and gastrointestinal parasites of small terrestrial mammals, found in Muni-Pomadze ramsar site and the grtassland in the University of Ghana, Legon main campus grassland ecosytem.

1.3 Study Objectives

The objectives for the study are as follows:

1.3.1 General objective

The main aim of the study is to investigate parasites in small terrestrial mammals in Muni-Pomadze Ramsar site and the University of Ghana main campus.

1.3.2 Specific objectives

1. To identify small mammal species in the study areas

2. To estimate prevalence of gastrointestinal parasites in the small terrestrial mammals.
3. To characterise ectoparasites in the small mammals caught from the study sites.

CHAPTER TWO

LITERATURE REVIEW

2.1 Ectoparasites of small mammals

Ectoparasites are organisms that live or infect only the superficial layers of the skin of a host, from which they derive their sustenance (Zeibig, 2013). Although the term ectoparasites can broadly include temporarily blood-sucking arthropods such as mosquitoes, this term is generally used more narrowly to refer to pathogens such as ticks, fleas, lice, parasitic flies and mites that attach or burrow into the skin and remain there for relatively long periods of time (Zeibig, 2013). Several ectoparasites use small mammals as hosts for their survival. A few of these ectoparasites are discussed below.

2.1.1 Tick infestation

Ticks (Acari: Ixodida) are external parasites of terrestrial vertebrates that feed on blood and tissue of their hosts; and are considered to be the second only to mosquitoes worldwide vectors of human diseases, but they are regarded as the most prevalent vectors of disease-causing pathogens in domestic and wild animals (De la Fuente, 2008). There are three families of ticks, two of which are considered to be of veterinary and medical significance- Ixodidae (hard ticks) and Argasidae (soft ticks), which are composed of 702 and 193 described species, respectively (Guglielmone *et al*., 2010; Nava, Guglielmone and Mangold, 2009). Ixodidae possess the most complex feeding biology of all hematophagous arthropods (Sojka *et al*., 2013). Ticks of the *Rhipicephalus* genus (Acari: Ixodidae) are obligate hematophagous arthropods, and a model for the study of ectoparasites vector-host interactions (Dantas-Torres, 2008; Tabor *et al*., 2017; Antunes *et al*., 2018). Ticks are the

obligate blood-feeding ecto-parasites of many hosts, including mammals, birds, and reptiles, and are also vectors for several bacterial, parasitic, or viral pathogens and after mosquitoes, ticks are the second most common arthropod pathogen vector (De la Fuente *et al*., 2008). Ticks are obligate hematophagous Acari that parasitize vertebrate animals and occasionally bite humans (Parola, 2004). They play a role as vectors and/or reservoirs for a variety of pathogenic microorganisms (e.g., bacteria, protozoa, viruses, and helminths) (Tahir *et al*., 2020). Ticks become infected with these microorganisms by feeding on infective hosts (human or animal) and, after a cycle of biological development, later inject the microorganisms into the new host during their subsequent blood meal (Jongejan and Uilenberg, 1994; Mulenga, 2014). Ticks and tick-borne diseases (TBDs) threaten livestock health, welfare and productivity in the whole of sub-Saharan Africa (Larusso *et al*., 2016). Ticks can easy multiply due to the climatic conditions of this region, and in general it is difficult to control, causing high infestation of pastures, reduced milk production, poor feed conversion, and consequently poor weight gain, and elevated cost in diseases treatment (Pazinato *et al*., 2014).

They also play a primary role as vectors of pathogens for animals, resulting in a multitude of infectious diseases worldwide (Brites-Neto, 2015). Ticks primarily feeding on companion animals may feed on humans in the absence of preferred hosts, resulting in the incidental transmission of tick-borne pathogens to humans (Jongejan and Uilenberg, 2004). Ticks are of great medical and veterinary significance due to their ability to transmit several pathogenic microorganisms to human and animal hosts (Barker, 1998; Dantas-Torres, 2010). These tick-borne pathogens are transmitted to other ticks and hosts associated with ticks by transovarial and transstadial transmission (Dantas-Torres, 2008; Rynkiewicz, 2015). Several tick-borne pathogens from ticks infesting dogs that are well-documented include *Anaplasma phagocytophilum* which is associated with human and canine granulocytic anaplasmosis,

Babesia spp. causing canine babesiosis, *Coxiella* spp. causing Q-fever in humans, *Ehrlichia canis* associated with canine ehrlichiosis, as well as *Rickettsia* spp. causing African tick bite fever, Mediterranean spotted fever, and Astrakhan fever (Mtshali *et al*, 2015).

Of the 18 TBPs reported from domestic animals and/or ticks, about 50% were zoonotic pathogens (Jimale, Wall and Otranto, 2023). Crimean Congo hemorrhagic fever (CCHF) virus can be transmitted to humans mainly via the bite of infected ticks or direct contact with the blood/tissue/ fluids of the infected animal (Emadi Kochak *et al*, 2003). CCHF infections in humans usually give rise to severe, acute hemorrhagic fever with a mortality rate of 10-50% (Mokhtari and Faraji, 2017). Hard ticks play a key role in the circulation of the virus in nature, serving as both the reservoir and carrier of the CCHF virus (Mehravaran *et al*, 2013). Tick-borne encephalitis virus (TBEV), belonging to the *Flavivirus* genus of the Flaviviridae family, causes mild or moderate febrile illness and fatal encephalitis with sequelae in humans (Yoshii, 2019). Changes in climate, habitat, and hosts have been found to be major drivers of ticks and TBDs expansion and invasion (Ogden and Lindsay, 2016).

The species which transmits *Borrelia recurrensis* which cause relapsing fever in *Homo sapiens*, the *Ornithodoros,* and various *Argas* species, especially those in chicken and other birds attack human when their natural host is unavailable (Adelson *et al*., 2004). Ticks [i.e. *Rhipicephalus decoloratus*; *Rhipicephalus annulatus*; *Rhipicephalus guilhoni*; *Rhipicephalus geigyi*; *Hyalomma truncatum*; *Amblyomma variegatum*; *Rhipicephalus simus* Group; *Rhipicephalus turanicus*; *Rhipicephalus sanguineus* (sensu lato); *Hyalomma rufipes* and *Rhipicephalus lunulatus*] include the vectors of pathogens of veterinary and zoonotic importance (i.e. *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Babesia* spp. and *Theileria* spp.) (Larusso *et al*., 2016). According to Chang *et al*., (2001), during a study several pathogenic *Bartonella*, which includes *B. henselae, B. quintana, B. washoensis, B. vinsonii* and *B. bovis* in about 19.2 % of questing adult *I. pacificus* ticks that were collected: in a

different study, *Bartonella spp*. were found in both nymph and adult *I. pacificus* ticks, in addition to *D. variabilis* and *D. occidentalis* ticks. Almost around this same time, *Bartonella spp*. from *I. scapularis* deer ticks that were collected from the household of two patients that were both simultaneously infected with *B. henselae* and *Borrelia burgdoferi* detected from their spinal fluid (Eskow *et al*., 2001), and from 34.5 % of field collected *I. scapularis* in the United State of America, New Jersey (Adelson *et al*., 2004). *Bartonella spp*. DNA has since been detected in ticks all over the world, which includes *Carios kelleyi*, *I. persulcatus*, *Dermacentor reticulates*, *Hyalomma longicornis*, *Rhipicephalus microplus*, *Haemaphysalis laechi*, *H. rufipes*, and *H. longicornis* (Loftis *et al*., 2005). *Rhipicephalus sanguineus* are also anthropophilic and vectors of pathogens to humans (Parola *et al*., 2008).

Many exposures to zoonotic diseases occur in the home through direct or indirect contact with pets, agricultural animals, or livestock (Aenishaenslin *et al*., 2013). Zoonoses require close contact with domestic animals or their excretions and frequently involves a violation of good hygiene practices (Rabinowitz, Gordon, Odofin, 2007). Some of these pathogens are zoonotic and of public health concern (De la Fuente *et al*., 2008). Often, clinical illness affects both the person and the animal, but sometimes the animal may appear healthy only to have a subclinical infection or colonization that can lead to illness in a person (Day, 2016). Ticks transmit more pathogen species than any other group of blood-feeding arthropods worldwide (Keirans and Durden, 1998). In sub-Saharan Africa, population growth and grazing pressure, exacerbated by socioeconomic pressures and human-induced environmental changes, are resulting in increasing exposure of domestic animals and humans to ticks and TBPs (Heylen *et al*., 2021). The effect of ticks and tick-borne pathogens (TBPs) on the economies and livelihoods of people dependent on livestock is particularly pronounced in Africa (Sahil *et al*., 2004).

Patients that are infected with ticks usually show skin reactions to the part of the site of the skin bitten, which may include inflammatory infiltration of the tissues, hemorrhage, local hyperemia and oedema (Zeibig, 2013). Sometimes there is serious tissue reactions as well as secondary infections may also occur when the mouth parts of the tick get stuck in the skin of the host after a trying to remove the whole tick and when certain tick species like *Dermacentor* are introduced into the host, tick paralysis may occur. In a situation where the tick is not removed, a toxemia may occur which may lead to death (Zeibig, 2013). Babesiosis, a disease caused by tick-borne intra-erythrocytic protozoan parasites that live within the red blood cells is a well-known disease which infects some small and large terrestrial mammals including cattle and sheep (Criado Fornelio *et al*., 2003; Hunfeld *et al*., 2008).

The adult tick has eight legs (four pairs), has two pairs of mouthparts but no antenna is present, just like all arthropods. They belong to the order Ixodida comprising of the family Ixodidae which are hard bodied, and family Argasidae which have soft bodies. Both are somehow having oval shape. The head of a tick, the thorax and abdominal regions are fused together appear as a single structure (Zeibig, 2013). They are dioecious (the sexes are in separate individuals). Two major morphological differences exist between soft and hard ticks. Both of the types possess a capitulum at the anterior region of the body (Zeibig, 2013). This structure can be seen on the dorsal region of hard ticks, but cannot be seen on the dorsal region of soft ticks because it is positioned ventrally (Zeibig, 2013). On hard ticks, a structure called scutum is located posterior to the capitulum. Soft ticks lack scutum and possess a leathery outer surface instead. A typical tick ranges from 6-8 mm long in size (Zeibig, 2013).

Ticks undergo complete metamorphosis, their life cycle has four (4) morphological stages: eggs, larvae, nymph and adult or imago of separate sexes (Fig. 1). Its life cycle usually ranges

from 1-2 years, depending on the season the hatching occurred (Zeibig, 2013). They can transfer many microorganisms to their offspring, ensuring that the infection is sustained. Larvae which are motile emerge from the eggs, then move to areas like the lamina of grass as well as twigs. The larvae eagerly jump from the resting place onto the first viable host that passes by. Once it attaches itself to the host it finds, its larvae feed on blood meal for some few days and then fall off the host and back to the ground where it molts into nymph. The nymph for the second time will migrate to possible areas where it can possibly locate a host to wait for another host. After it succeeds in attaching itself to a second host the process the larval stage went through with the first host is repeated. After falling back on the ground for the second time, the nymphs then molt and metamorphose into adult (Zeibig, 2013). Ticks of the family Ixodidae were often found attached to the tail; eggs, larvae, nymphs and adults of Listrophoridae and Glycyphagidae were attached to hairs (Estébanes-González and Cervantes, 2005).

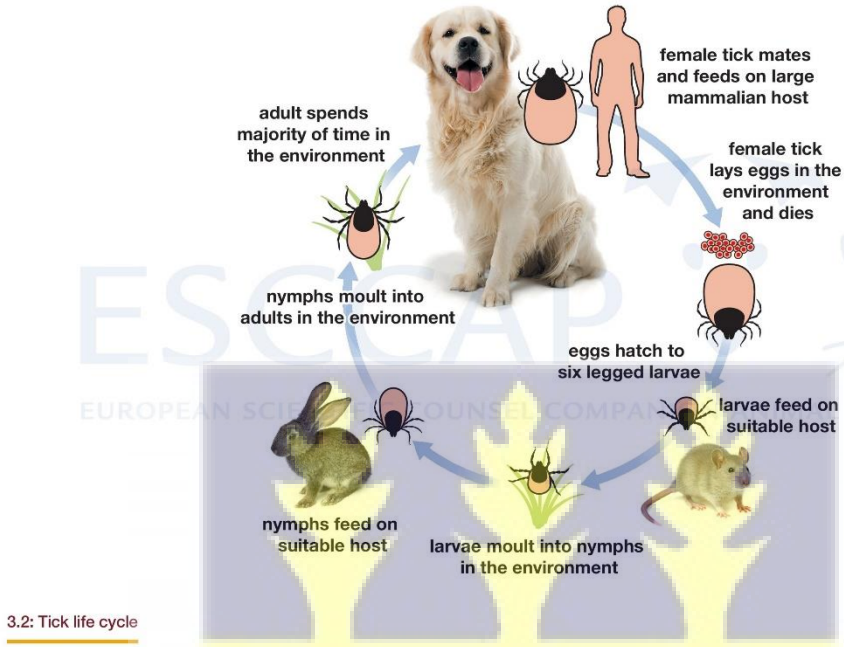

Figure 1: Life cycle of a tick (Downloaded from https://www.esccap.org/uploads/docs/b3rbhl5x_3.2_Tick_life_cycle_WM.pdf)

2.1.1.1 Distribution of ticks

Ticks can be found throughout the world; hard ticks are responsible for transmission of bacterial, viral, and rickettsial diseases. Hard ticks like *Ixodes spp*. (deer ticks) have a broad geographical range covering most of North America (Zeibig, 2013).

The ticks that belong to genus *Amblyomma*, are usually parasites of both large and small mammals which can be found in tropical as well as sub-tropical area of sub-Saharan Africa and America. Those that belong to parasitize cattle are *Boophilus* and can be found in tropical to the temperate regions of the world (Ärzte-Verlag, 2013). Ticks that belong to the genus

Dermacentor mainly parasitize large and small mammals throughout the tropical regions of Canada to Latin America. Those that belong to the genus *Haemaphysalis* mainly parasitise birds and small mammals that are found all over the world (Acha, 2003). Those that belong to the genus Hyalomma are mainly parasites of domestic animals that can be found in the Old World below the forty-fifth parallel North, but Ixodes are parasites of large and small mammals as well as birds that can be found throughout the world (Acha, 2003).

Human are usually infested by twelve species that belong to the family Argasidae (comprising of *Ornithoodoros* and *Argas*), and twenty-two species of the family *Ixodidae* (seven of genus Dermacentor, four of genus *Amblyomma*, three of genus *Haemaphysalis*, six of Ixodes and two of *Hyalomma* (Estrada-Pena and Jongejan, 1999). *Amblyomma*, *Dermacentor variabilis*, *Ixodes scapularis*, *Dermacentor andersoni*, *Ixodes pacificus*, and *Ornithodoros* were the species of ticks that could be found all over the United States of America (Merten and Durden, 2000).

2.1.1.2 Control of ticks

The widely recommended remedy for tick infestation is the removal of the tick, which is usually enhanced by placing a few drops of chloroform or ether on the tick's head and then pulling it out of the skin of the host, by using forceps to hold firmly the anterior region of the tick (Ärzte-Verlag, 2013). It is advisable to remove the whole tick because secondary infection and severe tissue reactions may occur due to mouth parts that get stuck in the skin of the host (Ärzte-Verlag, 2013). Effective and environmentally friendly control methods, such as vaccines among other interventions, are required to control tick infestations and tick-borne diseases (De la Fuente *et al*., 2017; De la Fuente, 2018; Molaei *et al*., 2019, Wikel, 2018).

It is difficult to eradicate ticks but several measures can be employed to reduce the possibility of being infected. It is advisable to avoid entering areas that are infested with ticks. In the case where one must be in such places, it is very necessary to use tick repellants and protective clothing. In order to help protect individuals from deadly rickettsial infections that are transmitted by ticks, a prophylactic vaccination has been developed. It is also advisable to remove ticks carefully as early as possible in order to break the disease transfer, since it may take hours to days for the transfer of infections during a tick bite (Ärzte-Verlag, 2013). In a study conducted by Pazinato *et al* in 2014, it was discovered that chemicals that are produced by combining two or three active ingredients like chlorpyrifos, cypermethrin, piperonyl, citronella; but those made from just one active ingredient like amitraz as well as diazinon showed lower efficacy against ticks. Commercial drugs that has a single active ingredient even though may be cheap to the farmers, promotes a faster tick resistance (Pazinato *et al*., 2014). Antibiotic use in animals is the primary cause of selection and spread of antimicrobial resistance in animals (Sundsfjord and Sunde, 2008). During the blood feeding process, ticks confront diverse host immune responses and have evolved a complex and sophisticated pharmacological armament in order to successfully feed (Liu and Bonnet, 2014). This includes anti-clotting, anti-platelet aggregation, vasodilator, antiinflammatory, and immunomodulatory systems (Francischetti, 2009).

2.1.2 Mite infestation

Mites although visible to the naked eye, are very small. Whichever species you find, they are oval in shape and their sizes ranges between 0.1-0.4 mm. In a specimen, microscopic examination is needed for confirmation (Zeibig, 2013). The majority of Cheletosomatini spp are obligate predators residing in wing quills; however, mites from the genus Picocheyletus

or Metacheyletia are likely parasites rather than predators in quills (Bochkov and Skoracki, 2011; Skoracki, 2016).

The adult mite infests the human or any other host burrow directly into the skin, the sebaceous glands or the follicles of the hair, and hence lives there. During this period, they lay eggs in the burrow which hatches and later matures from the larval into adult (Figure 2). The newly developed mites can now begin to continue the spread of the infestation. It takes approximately a fortnight (two weeks) for a mite to develop from the egg to adult stage. Mites can spread very fast especially in overcrowded conditions like refugee camps, schools, markets, hospitals, children homes, etc. but the transfer of mites from one person to another usually requires the two people to be in a prolonged close contact with each like in the case of a couple, sexual partners or those living in a household. Parasitic mites of humans include chiggers (i.e. Trombicula autumnalis), human scabies (Sarcoptes scabiei) and Demodex mites (Litwin *et al*., 2017). A total of 140 species or subspecies have been identified worldwide in 11 orders of mammals including humans (Zhao *et al*., 2012). Human Demodex have been found in nearly all age and racial groups (Chen and Plewig, 2014); and every human being carries a colony of 1000 to 2000 *Demodex* mites (Gutierrez, 2000). Species of the family Laelapidae were usually present in the dorsal region distributed among hairs, but species of Trombiculidae were attached to the skin in different regions of the body (Estébanes-González and Cervantes, 2005). Mites of family Myobiidae were found in the dorsal region of the host attached to the skin (Estébanes-González and Cervantes, 2005). Ptyctimous mites (Acari: Oribatida) are doubtlessly one of the best-known taxonomic groups of oribatid mites worldwide (Niedbała *et al*., 2023). There are two species of mites that parasitize humans: *D. folliculorum*, and *D. brevis* (Litwin *et al*., 2017). Quill mites of the subfamily Picobiinae (Acariformes: Prostigmata: Syringophilidae) are permanent and host-specific ectoparasites of birds (Sikora *et al*., 2023). Neotrichy is well known among phthiracaroid mites, where extra

setae appear usually on the notogaster and anoadanal plates (Niedbała *et al*., 2023). Parasitic mites are able to make optimal use of the host by colonising all available microhabitats; most live as ectoparasites, residing in the fur and on the skin, but others colonise the skin as mesoparasites and others the respiratory tract or digestive system as endoparasites (Kozina, Izdebska, and Rolbiecki, 2023). Breeding conditions favour infestations with atypical parasites that are passed from other mammals (neighbouring hosts) under favourable circumstances (Izdebska, 2001)

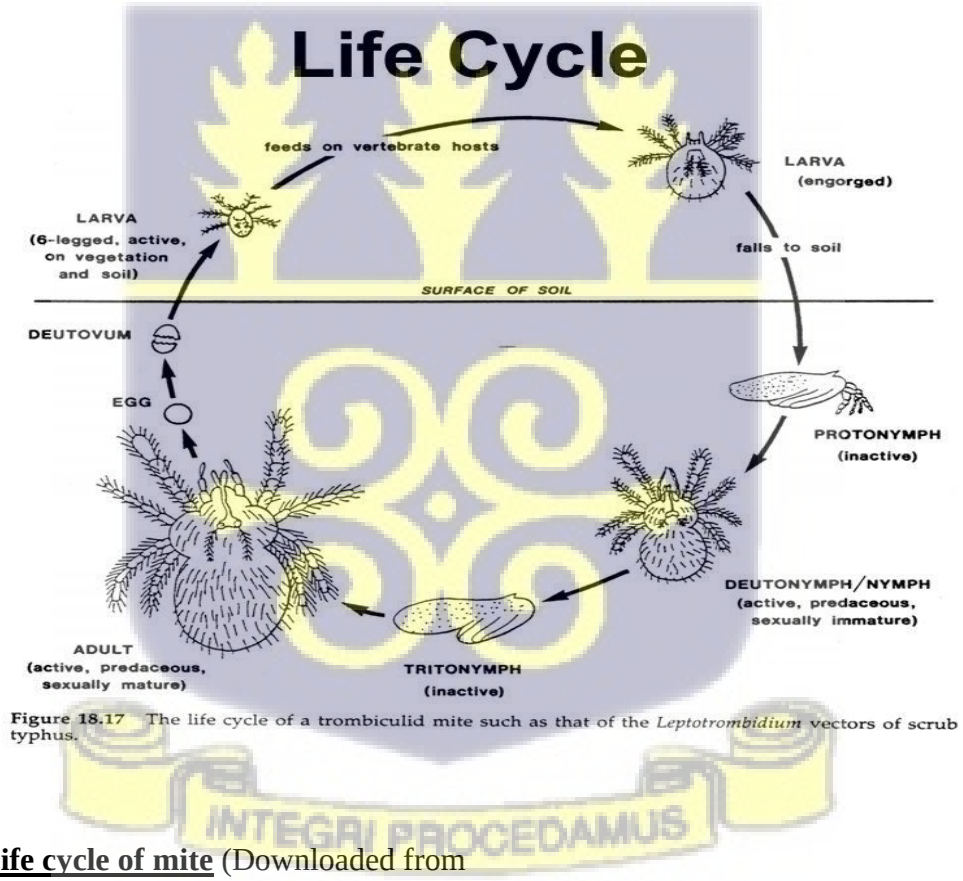

Figure 18.17 The life cycle of a trombiculid mite such as that of the *Leptotrombidium* vectors of scrub typhus.

Figure 2: Life cycle of mite (Downloaded from https://slideplayer.com/slide/3908792/13/images/28/Life+Cycle.jpg)

2.1.2.1 Distribution of mites

Mites are highly adaptable in the environment, with a worldwide distribution, and have separate forms through which they affect humans (Zeibig, 2013). *Trombicula akamushi*,

which is usually found in Asia, is the mite which carries scrub typhus, and *Liponyssus bacoti* may be a carrier of endemic typhus, rickettsial pox, and Q fever, and also, free living mites like house mites, species of *Dermotophagoides,* may be the cause of respiratory problems which is allergies in some individuals throughout the world (Zeibig, 2013). Some species of gamasid mites have been suspected to be the potential vectors or reservoir hosts of epidemic hemorrhagic fever and some other zoonoses, as well as the acariasis, which is caused by the direct parasitism of some gamasid mites within the human body and skin anaphylaxis by the mites' biting (Adler and Wills, 2003; Lance *et al*., 2004). Among wild animals,11 species of mites from the Ixodidae (Parasitiformes: Ixodida), Psoroptidae, Sarcoptidae (Acariformes: Astigmata), and Demodecidae (Acariformes: Prostigmata) have been found. (Kozina, Izdebska, Rolbiecki, 2023). In most wild animals, the mites do not cause any pathology; however, in domestic animals, particularly in dogs and cats, Demodetic mange can be deadly despite the fact that the mites are also present in most healthy dogs (Ravera *et al*. 2013).

Over a hundred different species of follicular mites have been morphologically described from a wide variety of animals, ranging from marsupials to placentals such as armadillos, bats, pigs, dogs, rodents, and primates (Smith *et al*., 2022). Their prevalence in humans is likely above 90%, where greater numbers and, thus, easier detection of mites are associated with an older host age and larger host pores; however, the density of mites in humans peaks with sebum production between 20 and 30 years of age (Foley *et al*.,2021). Existing data on the parasitic mites of mouflon in its various regions of distribution has been limited to a few mentions of the occurrence of ticks (Alonso, 2004).

2.1.2.2 Control of mites

Mites are all round, and therefore very difficult to avoid contact with, unless someone infested is known, hence prevention should primarily base on avoiding the spread to places that were initially affected and unaffected individuals, including making sure that all beddings, dresses and other belongings are washed in hot water and dried in a hot tempereatures (Zeibig, 2013). In the case where it is not possible to wash clothes within a short time, they should be placed in a plastic bag in order to stop the mites from spreading and finding another meal source since these mites will die within a few weeks without feeding (Zeibig, 2013). Keeping a good personal hygiene can also be employed to prevent mite infestation.

Several creams as well as lotions are prescribed for the treatment for mite infestation. Beddings, clothing, towels etc. must be cleaned and regularly disinfected and also washed in hot water. Using a hot cloth dryer to dry items is another way of treating mite infestation.

2.1.3 Flea infestation

Fleas are parasites of that infest vertebrates and can be found in various species forms on small terrestrial mammals. They can be found either in the immediate habitat of the host, or on the body of their host or both. The larvae can be independent feed on their own without being are parasitic. Species of fleas may be host specific or host-opportunistic (Marshall, 1981). Fleas are parasites of higher vertebrates and are most abundant and diverse on small mammals and usually alternate between periods when they occur on the host body and periods when they occur in their hosts' burrows or nests. (Krasnov *et al*., 2004). Fleas (Insecta: Siphonaptera) are obligate blood-feeding ectoparasites as adults and serve as vectors of several bacterial zoonotic pathogens, the most devastating being *Yersinia pestis*, the causative agent of plague (Eisen and Gage, 2012). The characteristics like small body size

that enables a flea to be densely populated on a host are the same characteristics that allow them to look for new host species to infest (Boris *et al*., 2004). Flea-borne trypanosomes of possible public health significance parasitized peridomestic rodents in several villages where the presumed flea vector was also found (Schwan *et al*, 2016). It thus appears advantageous for a flea species to exploit taxonomically close host species (Krasnov *et al*., 2004). behavioural defence against fleas is well developed among mammalian species, (Mooring *et al*., 2004). The Oriental rat flea, *Xenpsylla cheopis*, one of the most important urban vectors of the plague bacterium, *Y. pestis*, and the agent of murine typhus, Rickettsia typhi is widespread in southern Mali (Schwan *et al*., 2016).

Nosopsyllus fasciatus is commonly known as northern rat flea normally resides in the skin and fur of mouse, rat and human host (Boris *et al*., 2004). It can be found throughout the world and its main host is rodents. They are not shot-specific and therefore may attack any available bird or mammal in order to get its blood meal (Boris *et al*., 2004). They can survive without the host and therefore can be transmitted from housing and bedding. They are very mobile when on the host and hence can be found on the nesting material of host. Diagnosis is difficult since the adult can leave the host and larvae or eggs are difficult to find (Boris *et al*., 2004). Plague is a zoonotic bacterial infectious disease transmitted by fleas with reservoirs in rodent populations, in which fleas play a potentially fundamental role as bridge vectors to transmit the bacteria (*Yersinia pestis*) to animals and humans (Gallizzi *et al.*, 2008). Fleas undergo complete metamorphosis involving four stages: eggs, larva, pupa and imago (Fig. 3).

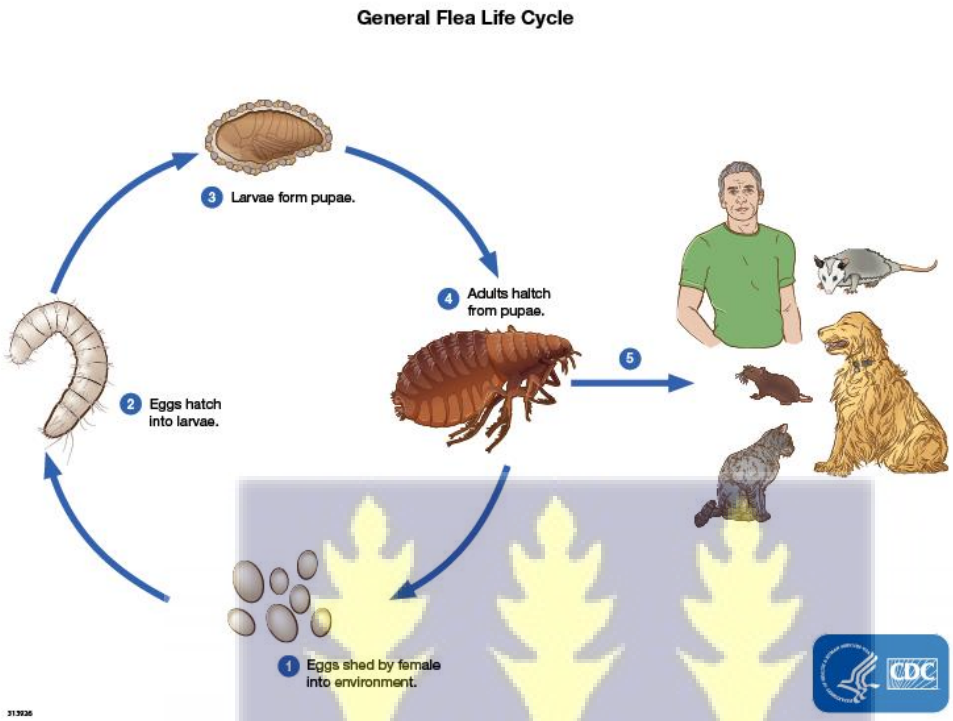

Figure 3: Life cycle of flea (Downloaded from https://www.cdc.gov/fleas/life_cycle_and_hosts.html)

2.1.3.1 Distribution of fleas

Fleas (Siphonaptera) are characteristic mammalian ectoparasites most abundant and diverse on small and medium-sized species with all stages of the life cycle spent off the host, except for the adults that feed intermittently on the host (Krasnov *et al*., 2005). The potential role of fleas as carriers and host-supported local reservoirs can help explain the persistent occurrence of plague (Gage, 2012). Transmission to humans sometimes occurs through contact with fleas that have fed on an infected small mammal or by skinning infected small mammals (Liang & Wang, 2011; Bai, Wang, and Si, 2014). There is an increasing awareness that these rodent-borne parasites have the potential to cause disease in people (Lun *et al*., 2009; Truc *et al*., 2013). Many flea species that were taken from rodents have been tested for the presence of

DNA of *Bartonella*. Species of *Bartonella*, including *B. elizabethae*, one that looks like *B. vinsonii*, *B. uintana*, *B. henselae*, *B. clarridgeiae*, one that looks like *B. schoenbuchensis*, *B. taylorii*, *B. tribocorum*, *B. queenslandensis*, and *B. rochalimae*, have been found in several gunus of fleas in several countries, including *Xenopsylla*, *Nosopsyllus*, *Sternopsylla*, and *Leptopsylla* (Winoto *et al*., 2005; Loftis *et al*., 2005; Li *et al*., 2007; Reeves *et al*., 2007; Tsai *et al*., 2010; Morick *et al*., 2010).

2.1.3.2 Control of fleas

In order to achieve optimum results, adult fleas should be killed to avoid re-infestation and in domestic animals, frequent vacuuming can be used to reduce infestation in the environment (Taylor, Coop and Wall, 2006).

2.1.4 Lice infestation

Lice are permanent obligate ectoparasites and highly host-specific; some of the parasites prefer specific body parts or positions on the host (Taylor, Coop and Wall, 2006). They are of veterinary interest a result of the damage they cause directly to their host, rather than being vectors. Some species of lice may serve as intermediate host to *Dipylidium caninum*-a tapeworm (Taylor, Coop and Wall, 2006). Heavy lice infestation is termed as pediculosis (Taylor, Coop and Wall, 2006).

2.1.4.1 Distribution of lice

Polyplax spinulosa is an ectoparasites that lives in the fur of rat and mouse and can be found worldwide. They are slender with its length ranging from 0.6mm-1.5mm with yellow-brown colouration, and the head bears a pair of prominent antennae which is segmented into five but has no eyes and no ocular points. They are rarely seen in laboratory rodents but common in

wild mice and rats and their infestation lead to restlessness, constant scratching especially behind the ears, and irritation. Both chewing and sucking lice have very similar lifecycle and the female lays about twenty to two hundred eggs within one moth life span. The eggs have white colouration and glued to the further or hair and are visible to the naked eye; the nymph that hatches is similar to the adult but smaller.

Adult lice lay eggs (nits) on or very near the host. Eggs of head lice may be found on in the hair shaft of the neck and head, but eggs of body louse found in chest hairs. Body and hair lice are ectoparasites with no wings and have three body segmentations comprising of head, thorax and abdomen. They have three pairs of legs having a clawlike feet extending from the thorax, which enables a louse to hold firmly on hair to avoid dislodging. It has a pair of antennae on its narrow head (Zeibig, 2013).

2.2 Endoparasites of small mammals

Endoparasites are parasites that live in the internal organs or tissues of its host. Parasites of the gastrointestinal tract including the following classe: Nematoda, Trematoda, and Cestodea) and protozoans (coccidians, flagellates) infect livetsock. Some of the fore mentioned organisms are of public health concerns because they can cause health challenges to animals and also cause diseases and can therefore reduce market value of animals and reduce income of farmers (Craig, 2018; Stromberg, 2006). Hence in rearing farm animals, it is important to incorporate accurate testing into management practices (Verocai *et al*., 2020.

2.2.1 Helminths

Parasitic helminths are estimated to range between 75,000 and 300,000 species and can affect humans, animals as well as plants (Taylor, Coop and Wall, 2006). Helminths of public health

importance are found in these higher taxa: Major: Nematoda (roundworms), Platyhelminthes (flatworms) which includes Trematodes (Flukes) and Cestodes (Tapeworms), and Minor: Acanthocephala (thorny-headed worms) (Taylor, Coop and Wall, 2006). Among parasitic animals, at least four species of flukes, a few species of cestodes, and approximately 20 species of nematodes, including roundworms, hookworms, threadworms, and lungworms, have evolved to undergo vertical transmission through breast milk in their vertebrate hosts (Chermette 2004; Foster *et al*. 2009; Boehm *et al*., 2015; Bezerra-Santos *et al*., 2020).

2.2.2 Protozoans

Protozoa are unicellular, eukaryotic (nucleus enclosed in a membrane), either free-living or parasitic organisms that exist independently with diverse forms of locomotion: by flagellum/flagella in *Trypanosoma*, by means of cilia in *Balantidium*, by pseudopodia in *Entamoeba*, and in the extracellular stages of *Eimeria*, there is no obvious means of locomotion but are capable of gliding movements (Ärzte-Verlag, 2013). Their sizes (approximately 1 to 300 µm) and shapes vary according to the various phyla and classes (Ärzte-Verlag, 2013). Life cycles are either direct, often including infective stages that are resistant to environmental influences (e.g., oocysts), or are indirect, including specific intermediate hosts, which may transmit the agent actively or passively after being, for example, ingested by the final host (Ärzte-Verlag, 2013). Lack of good sanitation as well as overcrowded areas enhances the spread of the disease coccidiosis. The oocysts are ovoid, yellowish or sometimes colourless, smooth, measures about 16 – 26 µm by 13 – 21 µm with elongated sporocytes that are ovoid in shape with a small Stieda body as well as residuum. At each end of the sporozoites are central nucleus having an eosinophilic globule. Some protozoans are *Trypanosoma cruzi*, *Balantidium coli*; *Giardia lamblia, Entamoeba histolytica* (Garcia, 2007)

2.3 Diagnosis of Ectoparasites

Arthropods act as important vectors in disease transmission of many parasitic diseases which are of human concern. Arthropods are invertebrates, consisting of a segmented body, several pairs of jointed legs, rigid exoskeleton, internal organs and body divided into head, thorax, and abdomen.

2.4 Diagnosis of Endoparasites

In the diagnosis of infections caused by parasites, laboratory diagnosis plays a critical a very important role. According to Sastry and Bhat, 2014, some of the techniques used to diagnose infections caused by parasites

- Morphological identification techniques involving either microscopy or macroscopy
- Molecular methods
- Culture methods
- Immunological methods
- Intradermal skin tests
- Xenodiagnostic techniques
- Animal inoculation methods
- Imaging methods.

2.4.1 Morphological identification techniques

In this technique, parasites by morphology either by microscopy or macroscopy. According to Sastry and Bhat (2014), stool sample can be seen directly by wet mount (either iodine or saline) or any other staining technique. Stool specimens should be collected in a clean, watertight container with a tight-fitting lid (Zeibig, 2013). It is advisable to do the collection

before treatment or any antiparasitic drugs are administered, and also close to when symptoms are imminent (Sastry and Bhat, 2014). The typical stool collection protocol consists of three specimens, one specimen collected every other day or a total of three collected in 10 days.) (Zeibig, 2013). It is recommended to examine semisolid stools within an hour, fifteen to thirty minutes when it is liquid stool, and when the stool is formed, examination should be done up to twenty-four hours after collection because trophozoites may appear as artifacts, degenerate or become non motile when stored for too long (Sastry and Bhat, 2014). To maintain the morphology of the parasitic eggs and cysts, ten percent polyvinyl alcohol or formalin can be used (Zeibig, 2013).

Perianal swabs, including NIH swab or cellophane tape, are good for the detection of Taenia species and *Schistosoma mansoni* eggs, and *Enterobius vermicularis* eggs that re deposited on the surface of the perianal skin (Sastry and Bhat, 2014). It is also very useful in detecting small intestinal parasite such as *Giardia intestinalis* as well as larvae of *Strongyloides stercoralis* (Sastry and Bhat, 2014).

2.4.1.1 Macroscopic examination

Mucoid bloody stool is found in invasive balantidiasis, intestinal schistosomiasis as well as acute amoebic dysentery (Sastry and Bhat, 2014). Dark stools may indicate bleeding high in the gastrointestinal tract, and fresh (bright red) blood most often is the result of bleeding at a lower level and in certain parasitic infections, blood and mucus may be present (Garcia, 2007). Helminth eggs may be found in any type of specimen, although the chances of finding eggs in a liquid stool are reduced by the dilution factor. Tapeworm proglottids may be found on or beneath the stool on the bottom of the collection container. Adult pinworms and Ascaris lumbricoides are occasionally found on the surface or in the stool (Garcia, 2007).

2.4.1.2 Microscopic examination

The most common procedure performed in the area of parasitology is the examination of a stool specimen for ova and parasites (Zeibig, 2013). Some drops of Lugol's iodine and saline are placed on the two ends of a slide, and then mixed with a small amount of faeces or stool using a stick in order to form a uniform smooth suspension (Sastry and Bhat, 2014). The mount is covered with a cover slip and examine under the low power objective (10X) which is succeeded by 40X high power objective. Cellular constituents such as erythrocytes (in the case dysentery), pus cells in the case of inflammatory diarrhoea may be seen (Sastry and Bhat, 2014). Another group of normal element that can be found are animal hair, bacteria, fat globules, pollen grains, muscle fibers, epithelial cells, air bubbles and starch grains which turns blue-black on reacting with iodine (Sastry and Bhat, 2014). Diamond shaped Charcot Leyden crystals which are products from the breakdown of eosinophils may be seen in sputum or stool of patients suffering from parasitic diseases like ascariasis, amoebic dysentery as well as some allergic diseases such as bronchial asthma (Sastry and Bhat, 2014). Larvae of helminthes as well as protozoan cysts and trophozoites can be seen (Sastry and Bhat, 2014).

According to Garcia, 2007, the types of iodine stains are;

- D'Antoni's iodine: one gram of Potassium iodide mixed with 1.5g of iodine crystals mixed with 100mL of distilled water
- Dobeil's iodine: two grams of potassium iodide and one gram of iodine mixed with fifty mL of distilled water
- Lugol's iodine: five grams of iodine crystals and ten grams of potassium iodide mixed with 100mL of distilled water

According to Garcia, permanent stained smears are required for diagnosing intestinal parasites accurately, by staining the internal structures of trophozoites and cysts, and the types include;

- **Modified acid-fast stain**

  According to Sastry and Bhat, this is use for identifying and detecting *Isospora belli, Cryptosporidium* and *Cyclospora*. Oocysts are stained red with carbol fuchsin while the background of the non-acid-fast background stains blue.

  Kinyon's cold method: This is where the faecal smear is fixed with methanol for about a minute, stained with Kinyon's carbol fuchsin for five minutes, and then rinsed with fifty percent ethanol and then followed by tap water. It is then stained with one percent sulfuric acid for two minutes to be decolorized. Washed with tap water and then alkaline methylene is used to counter stain for a minute.

  Hot method is where a thin smear of faecal matter is fixed with heat and then flooded with carbol fuchsin for a period of nine minutes. The slide is heated intermittently till the carbol fuchsin starts to steam. The slide is washed with tap water and then washed with five percent aqueous sulfuric acid to decolorize it, then washed with tap water and then counter stained for a minute with methylene blue.

- **Trichrome stain**

According to Sastry and Bhat, just like in the case of the iron-heamatoxylin staining method, the faecal smear is prepared, fixed and the treated with alcohol containing iodine. Trichrome solution is used to stain the slide for ten minutes and differentiated in acid alcohol (which is 1-part glacial acetic acid in ninety-nine parts of alcohol) for two to three minutes, followed by rinsing in absolute alcohol for several times and then dehydrated in absolute alcohol for two to five minutes. The stained smear is now placed in xylene for about two to five minutes and then mounted in Canada balsam and then a covered with a coverslip.

- **Iron-haematoxylin stain**

A thin smear of faeces is fixed using Schaudinn's solution for a period of fifteen minutes followed by immersion in seventy percent alcohol containing iodine, then in fifty percent alcohol for about two to five minutes each and then washed with tap water. The slide is then immersed in two percent aqueous ferric ammonium sulphate solution for about five to fifteen minutes and then washed in tap water for five minutes (Sastry and Bhat, 2014). This is followed by staining 0.5 percent aqueous haematoxylin from five to ten minutes followed by immersion in aqueous picric acid solution for ten to fifteen minutes and dehydrated by immersion for five minutes each in fifty percent, seventy percent, eighty percent and then ninety-five percent alcohol (Garcia, 2007). The stained smear is finally placed in xylene for two to five minutes and then mounted in Canada balsam and then covered with coverslip (Sastry and Bhat, 2014).

Concentration technique is very useful when the parasite output in faeces (larvae, eggs, cysts and trophozoites) is low and the use of direct faecal examination may not be able to detect the parasites, hence the need for concentrating the stool specimen (Sastry and Bhat, 2014). It is also a very useful method in assessing the people's response to treatment in epidemiological studies (Sastry and Bhat, 2014). Trophozoites are destroyed but cysts, larvae and eggs are recovered after the concentration is done. The commonly used concentration techniques are:

- **Sedimentation techniques**

The cysts as well as eggs settle down at the bottom after centrifugation. The three forms include Formal-ethyl acetate concentration technique, Formalin-acetone sedimentation technique and the Formalin-ether concentration technique (Sastry and Bhat, 2014).

- **Floatation techniques**

The cyst and eggs float on the surface due to difference in specific gravity (Sastry and Bhat, 2014). The Three main form include the Zinc sulphate floatation concentration technique, Sheather's sugar floatation technique (which is for *Cyclospora*, *Isospora* and *Cryptosporidium*), and Saturated salt (sodium chloride-NaCl) solution while saturated salt solution technique and formalin-ether are the two commonly used concentration techniques (Garcia, 2007).

Sedimentation technique principle involves using cenfugation to concentrate the stool specimen (Sastry and Bhat, 2014). The helminthic eggs and the cysts of protozoans are concentrated at the bottom of the tube because their gravities are higher than the medium in which they suspend (Garcia, 2007).

- **Formol-ether sedimentation technique**

Approximately four grams of faeces is transferred into a tube which contains ten mL of five to ten percent formalin, it is mixed thoroughly and then allowed to stand for thirty minutes (Sastry and Bhat, 2014). The mixture is then filtered into a fifteen mL conical centrifuge tube covered with two layers of gauze (Garcia, 2007). About eight mL of the filtrate is collected (about three to four mL formalin preserved). 0.85% saline (or five to ten percent formalin) is added almost to the top of the tube with the filtrate and then centrifuged at 500 x g for ten minutes (Garcia, 2007).

The supernatant is discarded and about 0.5-1 mL of sediment is suspended in the formalin or saline filled to the top of the tube and centrifuged at 500 x g for another ten minutes (Sastry and Bhat, 2014). The sediment is suspended again in five to ten percent formalin (filled half of the tube) and centrifuged. About 4-5 mL of ethyl acetate (or ether) is then added and the tube is then closed with a cork and then shaken vigorously in order to mix well. The cork is then removed and the tube is centrifuged for ten minutes at 500 x g (Sastry and Bhat, 2014).

Four layers are formed with the top consisting of ether, second is made of debris, the third is clear layer of formalin and the last being sediment (Zeibig, 2013). The debris is removed from the side of the tube using a glass rod and then the supernatant is discarded (Sastry and Bhat, 2014). Using a pipette, remove the sediment and then make an iodine or saline mount for examination under a microscope (Sastry and Bhat, 2014).

Floatation Techniques use the principle of suspending specimen in a medium which has a higher density than that of the cyst of protozoa or helminthic eggs, hence the cyst and eggs float on the top and are collected by placing a glass slide on the surface of the meniscus at the top of the tube and then covered with a coverslip before examining it under the microscope (Garcia, 2007).

Zinc sulphate floatation concentration technique Strain the specimen through a filter containing a singlelayer thickness of gauze into a conical centrifuge tube. Fill the tube with saline and centrifuge for 10 minutes at 500 × g (1500 rpm). Decant the supernatant. If the supernate is cloudy, repeat this step for a second wash. Resuspend the sediment with 1-2 mL of zinc sulfate solution. Fill the tube with additional zinc sulfate to within 2-3 mm of the rim. (The zinc sulfate must have a specific gravity of 1.18-1.20.) Centrifuge for 2 minutes at 500 × g (1500 rpm). Allow the centrifuge to come to a complete stop. While the tube is in the centrifuge, remove one or two drops of the top film using a Pasteur pipette or a bent wire loop and place on a slide. Add a cover slip and examine microscopically. Iodine can also be added (Zeibig, 2013).

Egg quantification (Egg counting) Methods

This is done to ascertain the intensity of intestinal helminthic infection (Sastry and Bhat, 2014).

- **Direct smear counting method of beaver**

About two grams of faeces is mixed with a drop of saline on a slide to form a smear and then examined under the low power of a microscope (Sastry and Bhat, 2014).

The number of eggs in the two gram of faeces is counted mad the multiplied by the factor five hundred in order to calculate the number if eggs per gram of faeces, it is however more simple and accurate when performed by an expert (Sastry and Bhat, 2014).

- **Kato's cellophane tape**

The approximate number of eggs per gram of faeces during sedimentation technique or concentrated stool specimen can be calculated by Kato's cellophane tape covered thick smear examination (Sastry and Bhat, 2014).

Dilution egg counting or Stoll's method

Four grams of faeces and fifty-six mL of N/10 NaOH are thoroughly mixed in a calibrated Stoll's flask to form a uniform mixture (Sastry and Bhat, 2014).

Transfer 0.15mL of the mixture onto a slide kept over a mechanical stage and then examination is done under the microscope (low power) (Sastry and Bhat, 2014). Calculate the number of eggs per gram of faeces (N) by multiplying the count number (n) with hundred (Sastry and Bhat, 2014). Calculate the estimated daily output of eggs by multiplying the weight of twenty-four hour faecal sample by the number of eggs per gram (Sastry and Bhat, 2014). This method is primarily for formed eggs but the consistency of the stool can change the estimated egg per gram value (Sastry and Bhat, 2014). Hence, if the faeces is liquid or semi-solid, and not formed, then the egg per gram of faeces (N) is multiplied by the correction factor: Mushy diarrhoeic = 3N, Mushy stool (soft or pulpy) =2N and Mushy formed = 1.5N (Sastry and Bhat, 2014).

2.5 Rodents

The successful nature of this group of herbivores can be attributed to their small size, intelligence and rapid rate of reproduction (Johnson and Raven 2006). They belong to the largest order of mammals which is Rodentia, and they have upper and lower jaws bearing a single pair of incisors which continues to grow throughout their life time (Miller and Harley, 2001). They have chisel-like teeth for gnawing wood, grains and nut (Hopson and Wessells, 1993). Some typical examples are chipmunks, rats, squirrels, porcupines, lemmings, mice, woodchucks.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study site

The Muni-Pomadze Ramsar site is situated to the west of the coastal town of Winneba, in the Central Region of Ghana, approximately 56 km from Accra (Figure 4). It covers a total area of 90 km^2 surrounding shallow coastal Muni Lagoon, which has an area of 3 km^2 and a maximum depth of 1.5 m (Attuquayefio and Ryan, 1997). It has two protected areas, Yenku A and Yenku B Forest Reserves occupying almost 10% of the site, while the traditional hunting areas of the Efutu people cover nearly 10% of the site (Gordon *et al*., 2000). The approximate boundaries of the Muni-Pomadze Ramsar site lie between latitude *05° 19' - 5°27'N* and longitude *0°37' -0° 41' W;* and bounded by the stream which divide between the Mankwaafa and Brounye rivers on one side and then the Boaku river on the other side; bounded to the by the Gulf of Guinea and extends approximately 15 km inland and to the east, the stream divide between the Ayensu river and the Pratu stream (Gordon *et al*., 2000). The vegetation is partly forest and coastal savanna interspersed with thickets; the Ramsar site shares boundaries with human settlements, and thus, a level of interaction between humans and the animals that live in the site occurs (Attuquayefio and Ryan, 1997).

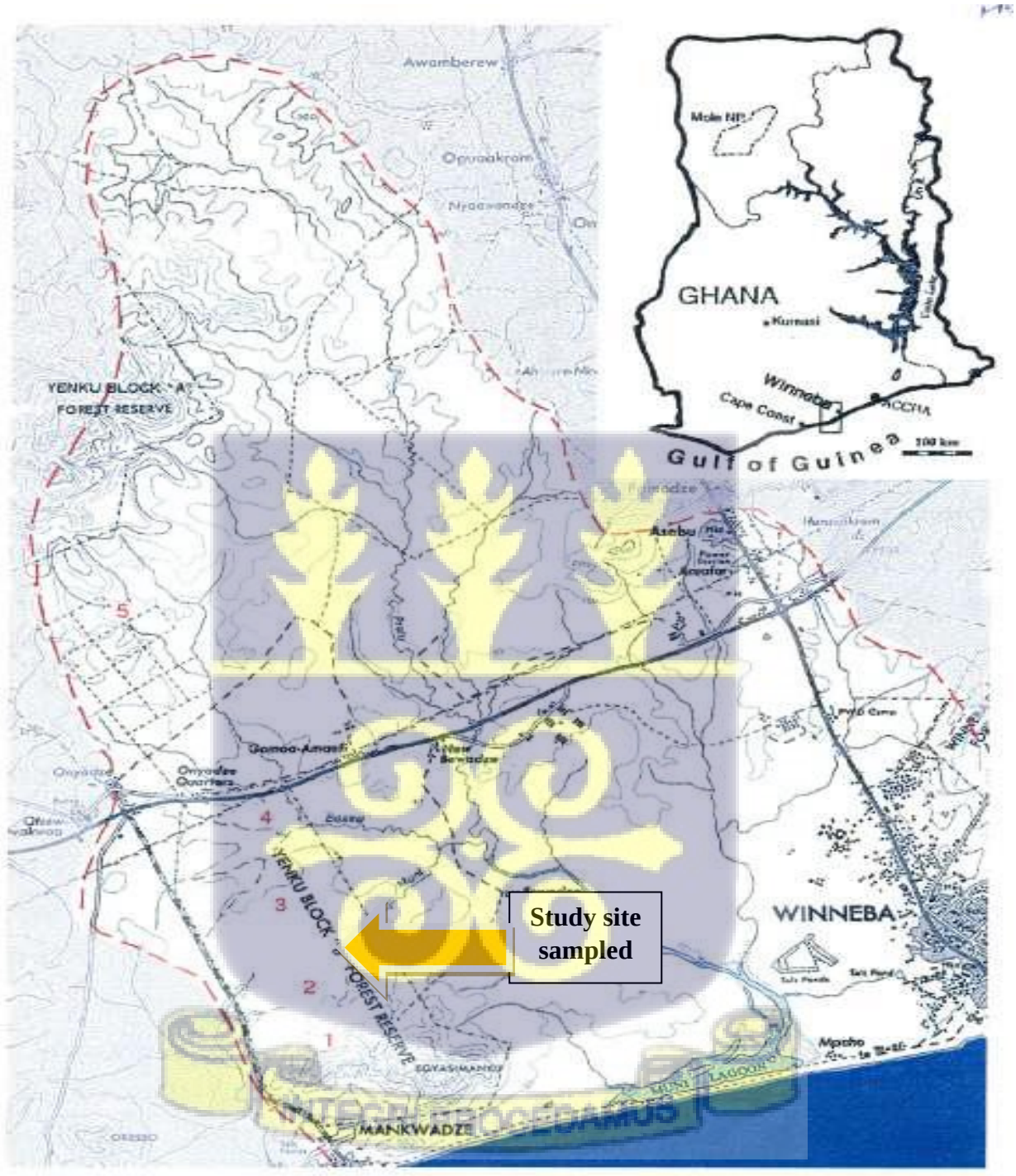

Figure 4: Location of Muni-Pomadze Ramsar site (Attuquayefio and Ryan, 1997)

The grassland in the University of Ghana is on the Legon main campus in the University of Ghana main campus, Accra (Fig. 5). It is used by several departments in the University of Ghana for research especially the Department of Plant and Environmental Biology. It has a

large natural vegetation. It is a famous laboratory for the Department of Plant and Environmental Biology and the Department of Animal Biology and Conservation Science, both of the University of Ghana. It is a scenic and excellent place where one can enjoy and traditional events such birds and butterflies watching. The farm also houses squirrels and lizards.

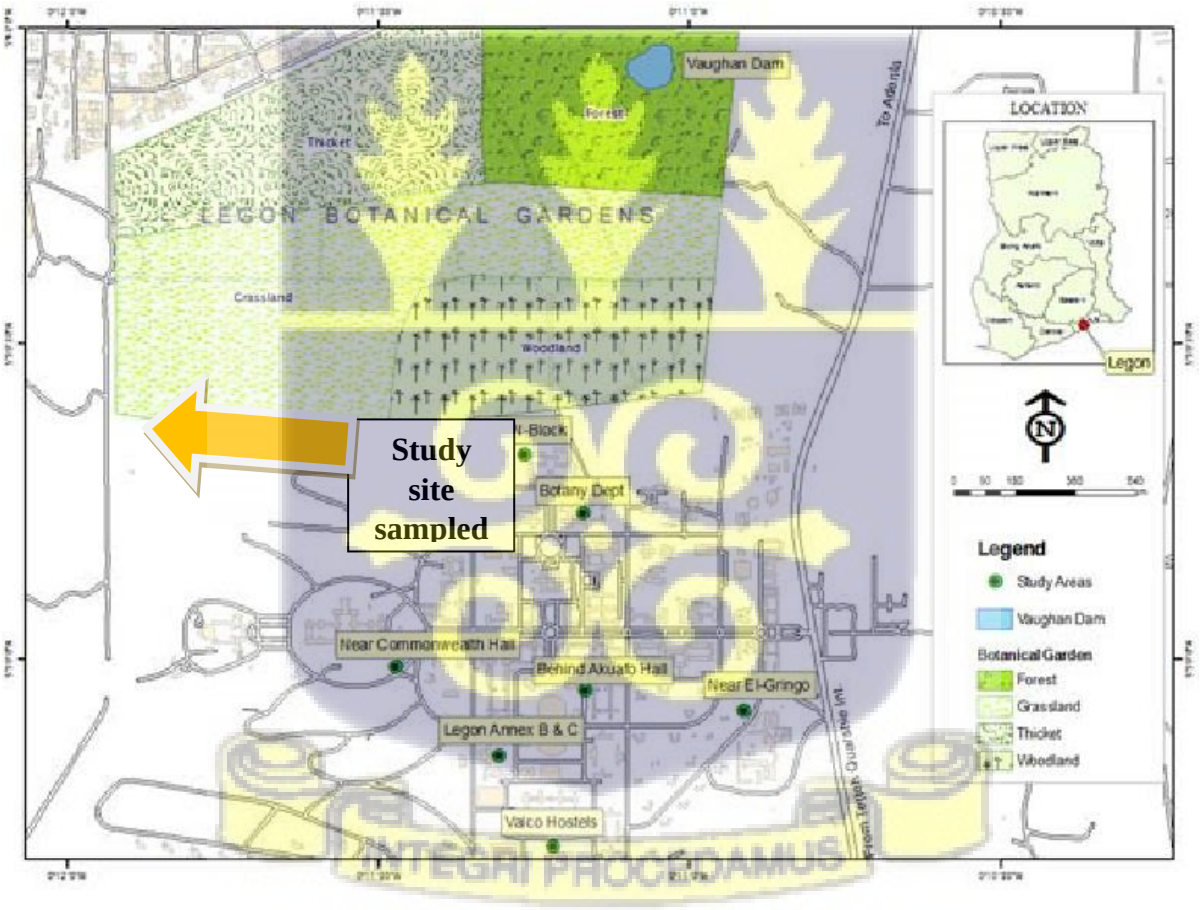

Figure 5: Map of University of Ghana main campus showing the University of Ghana Legon main campus

(Downloaded from https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.researchgate.net%2Ffigure%2FDistribution-of-transects-in-the-built-up-area-and-botanical-garden_fig2_261041768&psig=AOvVaw2dnoAIcuGJvAJYwjBPf8j8&ust=16858036640880

00&source=images&cd=vfe&ved=0CBEQjRxqFwoTCJC0x5jqpP8CFQAAAAAdAAAAABAE)

3.3 Study design

The study involved live-trapping of wild small terrestrial mammals along transects. Ectoparasites were collected from trapped animals and faecal samples collected for intestinal parasites identification. Blood samples from the tails were collected onto filter-paper (for haemoparasite identification) before they were released. The clipped tails also served as markers on the small mammals in case they are recaptured in the course of trapping.

3.4 Sampling method

The terrestrial small mammals were trapped using Sherman collapsible traps baited with fresh corn dough mixed with fresh groundnut paste, that were set along transects with inter-trap distance of 10m. Traps were set for six consecutive trap nights in November 2020. Captured animals were sexed (using the anal-genital distance, which is longer in males) and examined for reproductive condition (abdominal or scrotal testes in males and enlarged nipples, perofrate vaginas and pregnancy in females. The ectoparasites removed with the forceps into labelled containers with 70% ethanol prior to sorting and identification in the laboratory. The tip of the tail of a captured animal was clipped and blood drawn from it, and then released at or near the point of capture. Tail clipping mad eit possible to easily identify recaptured animals.

3.4.1 Collection of faecal sample

Faeces found in the trap after capture were collected using forceps into test tubes and stored in 70% ethanol prior to analysis in the laboratory. Where no faeces was found in the trap, some few minutes were allowed for the animal to defecate. Each faecal sample was serially labelled, the number and sex of the animal captured. The sample was stored in an ice chest container to preserve the eggs and also prevent larvae from hatching out. The sample was then transported to the laboratory for processing and analysis.

3.4.2 Processing of faecal sample by Modified Zinc Sulphate centrifugal floatation technique using physiological saline

Faecal sample was strained to get ethanol out. About 0.5 g of faecal residue was weighed into a petri dish. An amount of 14 ml of physiological saline was measured with a centrifuge tube or falcon tube. Saline was mixed with faecal residue in a beaker and then transferred to a centrifuge tube. It was then centrifuged for two minutes at 2000 rpm (rounds per minute). The supernatant was poured away. Zince Sulphate ($ZnSO_4$) solution was added to 14 ml mark on centrifuge tube and then it was shaken to dissolve. It was centrifuged for 2 mins at 2000 rpm. A portion of the sample from the surface was pipetted and two drops was put on two different slides (a drop for each slide) and a drop of iodine was added to each slide to stain. The slide was covered with a slip and examined under a Leica microscope with microscope, beginning at × 10 (Plate 1).

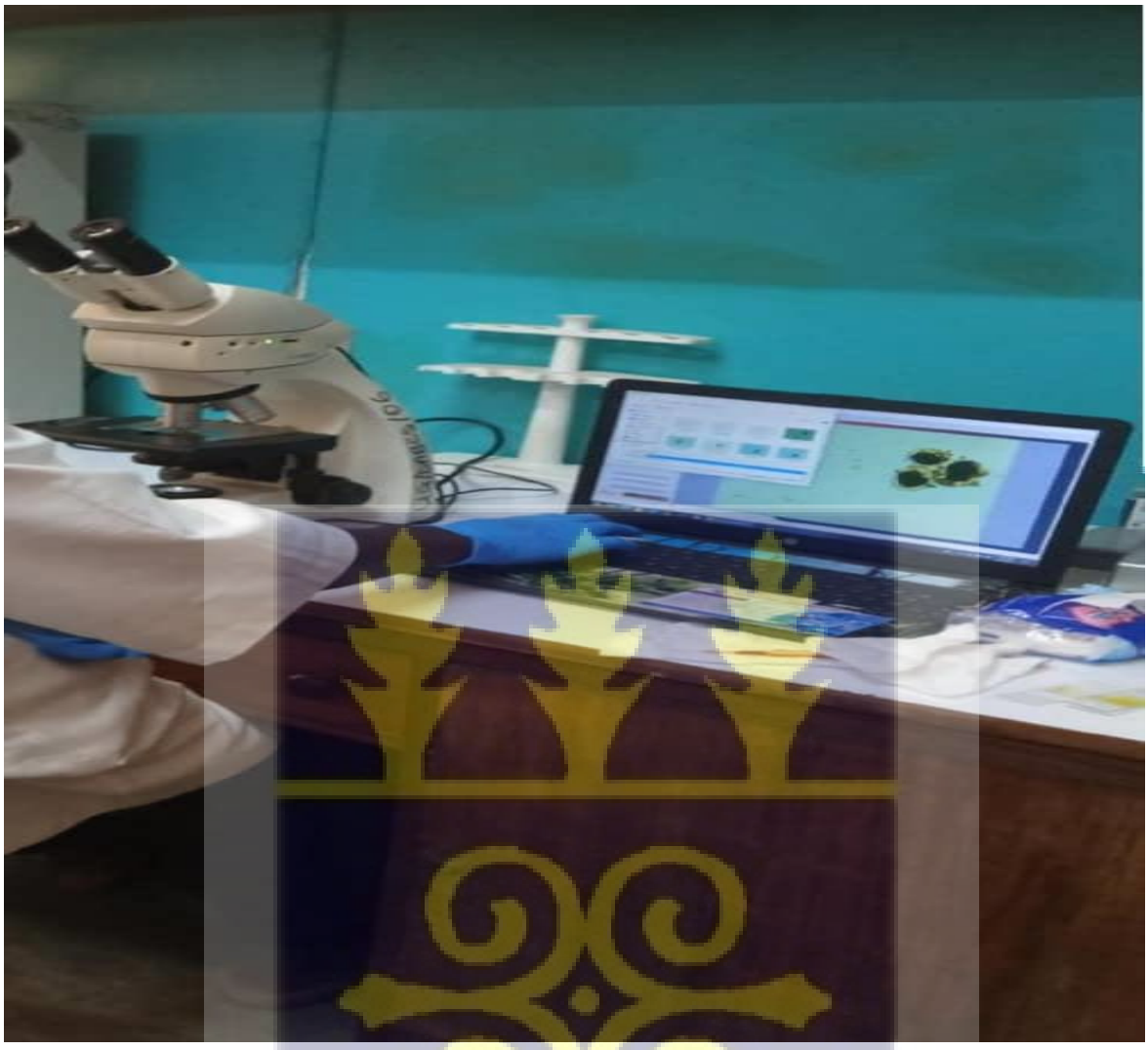

Plate 1: Identification of endoparasites under the microscope

3.4.3 Processing of ectoparasites for identification

Faecal sample was strained to get ethanol out. About 0.5 g of faecal residue was weighed into a petri dish. An amount of 14 ml of physiological saline was added to the sample and then observed under the dissecting microscope (Plate 2).

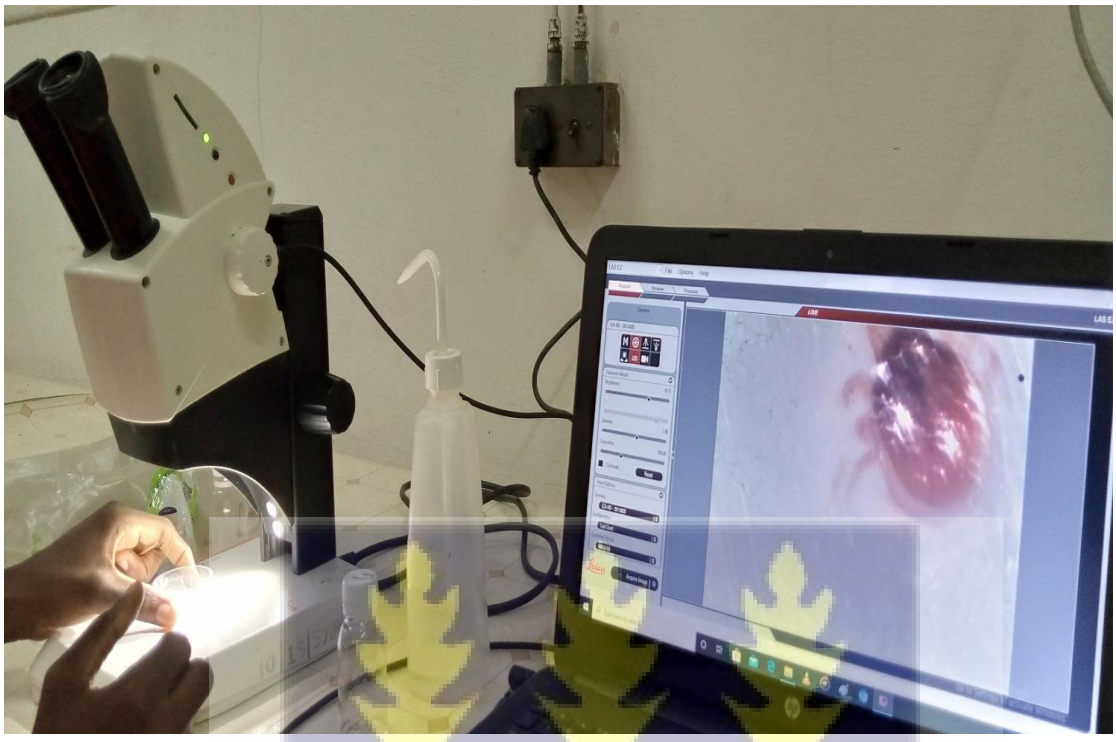

Plate 2: Identification of ectoparasites under the microscope

3.5 Identification of parasites

The keys provided in Veterinary Parasitology, pg 261=267 by Taylor M.A, Coop R.L, Wall R.L (2006), together with the WHO's online appendix was used to match the eggs to their corresponding parasites.

3.6 Data analyses

The data collected on individual small mammals were entered into Microsoft Excel spread sheet to create a database. The data analysis was done with JMP Pro 17; [CI=95%, p=0.05]. For each helminth species discovered, the prevalence, mean abundance, and mean intensity were calculated. The prevalence was estimated by dividing the number of infected host by the total number of host and multiplying the result by 100. The mean abundance was computed

by dividing the total number of host by the total number of parasites, while the mean intensity was computed by dividing the total number of parasites by the total number of infected hosts. The differences of parasitological matrices between male and female mammals were tested using chi square.

CHAPTER FOUR

RESULTS

4.1 Small mammals that were captured

A total number of 108 small mammal individuals were captured for the and examined for both ectoparasites and endoparasites (Plate 3). Twenty-nine *Mastomys natalensis* and five *Lemniscomys striatus* were collected from Muni-Pomadze ramsar site, while 74 were collected from the grassland in the University of Ghana, Legon main campus. 18 females, 11 are males of *Mastomys natalensis*, 4 males and a female *Lemniscomys striatus* were captured from the Muni-Pomadze ramsar site, 38 females and 36 males of *M. natalensis* were examined from the grassland in the University of Ghana, Legon main campus.

Plate 3: A picture of one of *Mastomys natalensis* from which samples were taken at Muni-Pomadze Ramsar site.

4.2 Prevalence, Mean abundance and mean intensity of intestinal parasites identified in the small mammals

The prevalence of *Ascaris* (63.5%) was highest in the grasssland in the University of Ghana, Legon main campus, but in Muni-Pomadze, *Ascaris* and *Fasciola* were both highest with 17.4% (Fig. 6 & 7). A total of nine species of intestinal parasites were ideintified including one Cestode, Nematode, Trematode and one Protozoa. The Cestode is *Hymenolepis* sp, the Nematodes are *Ascaris*, *Enterobius* sp., hookworm, *Strongyloides* and *Trichuris*, the Trematodes are *Dicrocoelium* and *Fasciola*, and the Protozoa *Balantidium* (Plate 4). Out of thirty-four small mammals captured in Muni-Pomadze ramsar site, eleven were infected with at least one of the following intestinal parasites: *Ascaris* sp. (17.6%), *Balantidium* (2.9%), *Dicrocoelium* sp. (2.9%), *Enterobius* sp. (2.9%), *Fasciola* sp. (2.9%), *Hymenolepis* sp. (2.9%), Hookworm (5.9%), *Strongyloides* sp. (17.9%), *Trichuris* sp. (8.8%) (Fig. 6). Out of seventy-four small mammals captured from the grassland in the University of Ghana main campus, 45 were infected with at least one of the following intestinal parasites, *Ascaris* sp. (63.5%), *Strongyloides* sp. (62.2%), *Trichuris* sp. (27%) (Fig. 7). The mean abundance and mean intensity of parasite infection were calculated for Muni-Pomadze (Fig. 8) and the and grassland in the University of Ghana, Legon main campus (Fig. 9). 64% of mammals in Muni-Pomadze had single infection rate while only 3% had quadruple infection (Fig. 10). 82% of small mammals have single infection with 14% double (Fig. 11). The mean intensity in male small mammals was higher in the grassland in UG than in Muni-Pomadze (Fig. 12). Prevalence and mean abundance was generally higher in the cropland mosaic farm in UG than in Muni-Pomadze (Fig. 13). Generally, mean abundance, mean intensity and prevalence were higher in females than males (Fig. 14). The mean abundance, mean intensity and prevalence of endoparasites that were only identified in Muni-Pomadze were also higher in females than in males, except for Hookworm (Fig. 15). There was no significant difference of

helminth infection between sex of mammals in Muni-Pomadze except for *Trichuris* [χ^2 =5.0135 df=1, p=0.0251] where infection was significantly higher in females; *Dicrocoelium* [χ^2 =4.1628, df=1, p=0.0413]; and *Hymenolepis* [χ^2 =4.2236, df=1, p=0.0399] where infection was significantly higher in males. In the grassland in the University of Ghana, *Strongyloides* was significantly higher in females [χ^2 =16.1023, df=1, p<0.0001].

Nine intestinal parasites species were identified including one Cestode, Nematode, Trematode and one Protozoa. The Cestode is *Hymenolepis* sp, the Nematodes are *Ascaris*, *Enterobius*, Hookworm, *Strongyloides* and *Trichuris*, the Trematodes are *Dicrocoelium* and *Fasciola*, and the Protozoa *Balantidium*. All the parasites identified are potentially zoonotic.

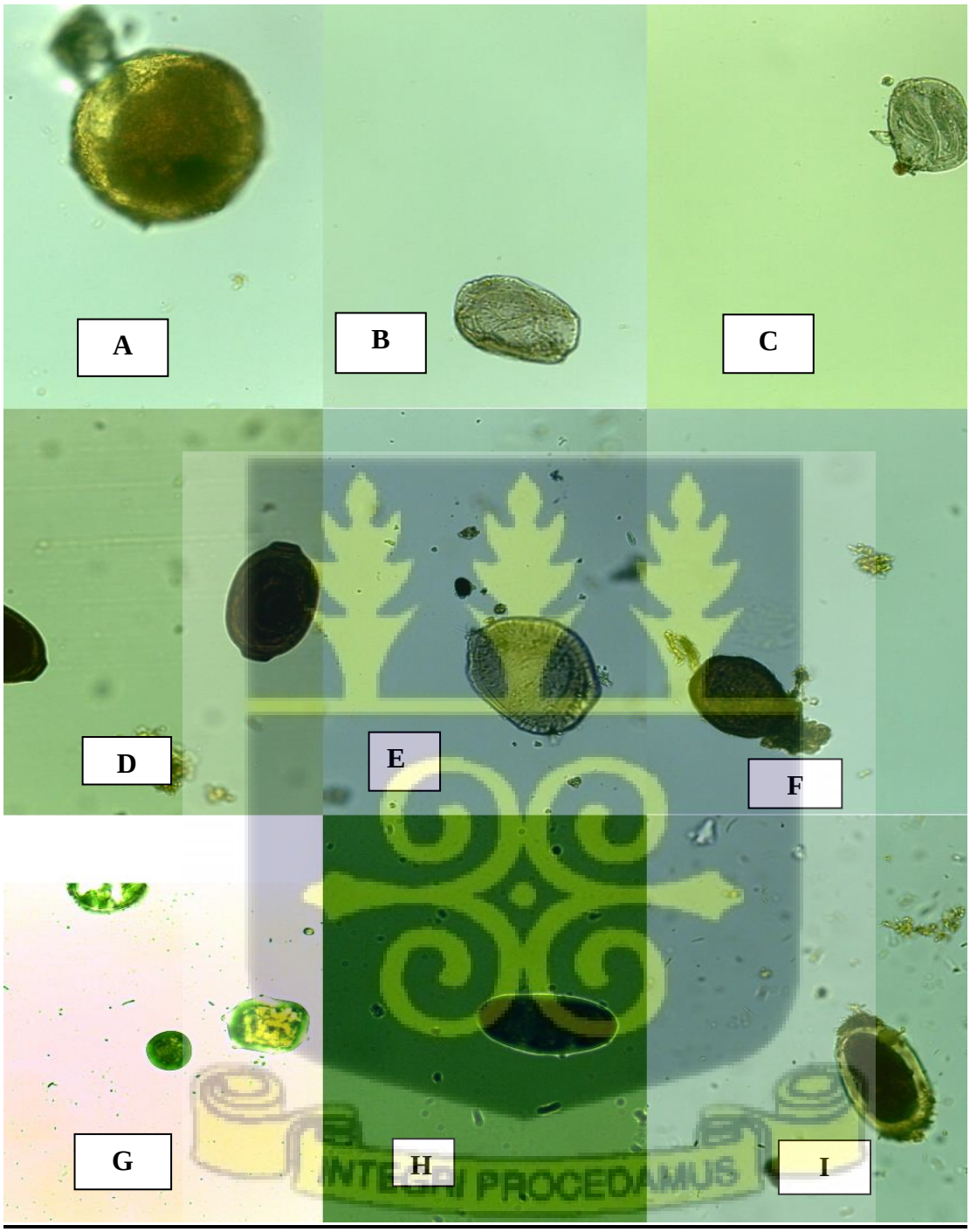

Plate 4: Pictures of some of the ova of parasites from the processed faecal samples of the small mammals

A= *Dicrocoecium* sp ; B= *Enterobius*; C= *Fasciola*; D= *Trichuris sp*; E= *Strongyloides*; F= *Balantidium*; G= *Hymenolepis;* H= *Ascaris*; I= Hookworm

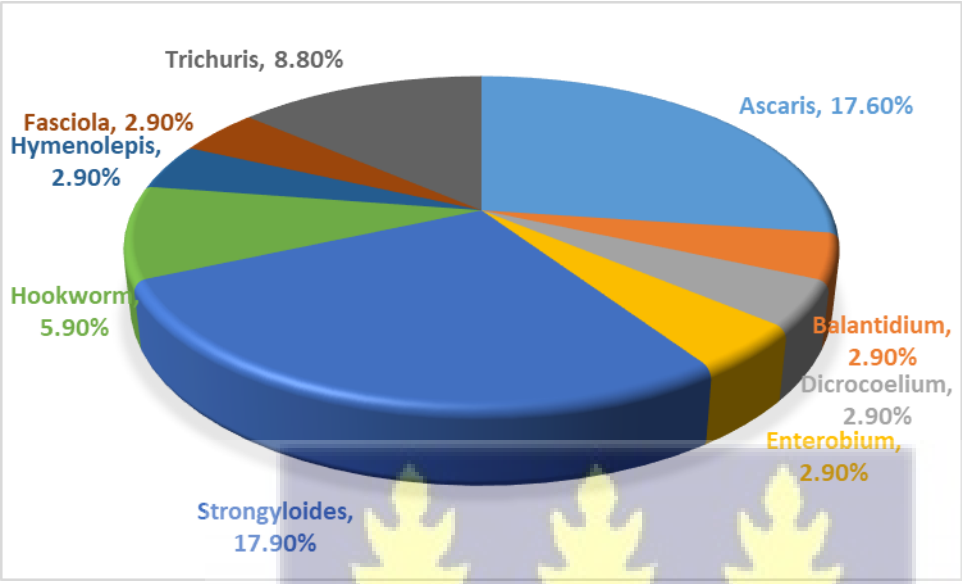

Figure 6: Prevalence of helminths identified in the faecal sample of small terrestrial mammals in Muni-Pomadze

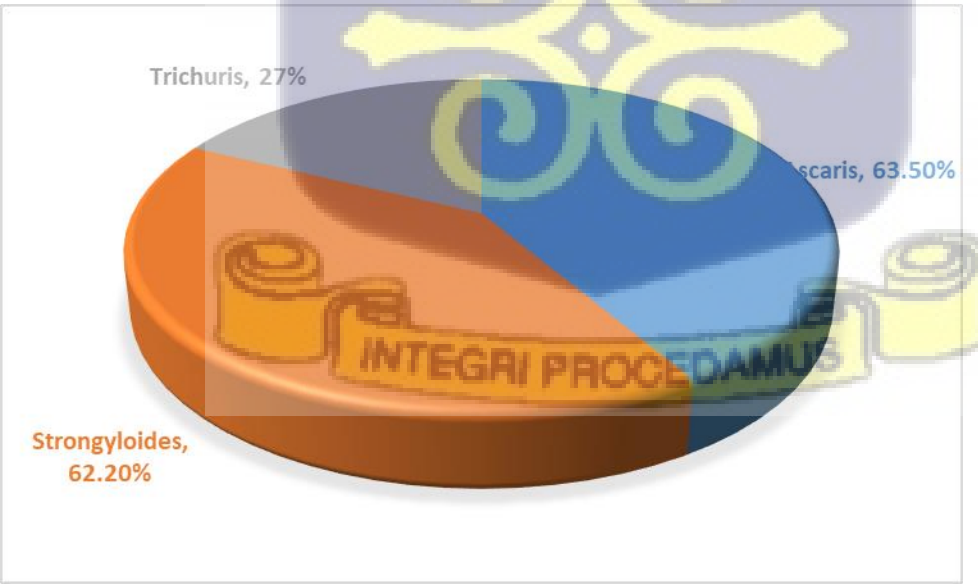

Figure 7: Prevalence of helminths identified in the faecal sample of small terrestrial mammals in grassland in The University of Ghana, Legon

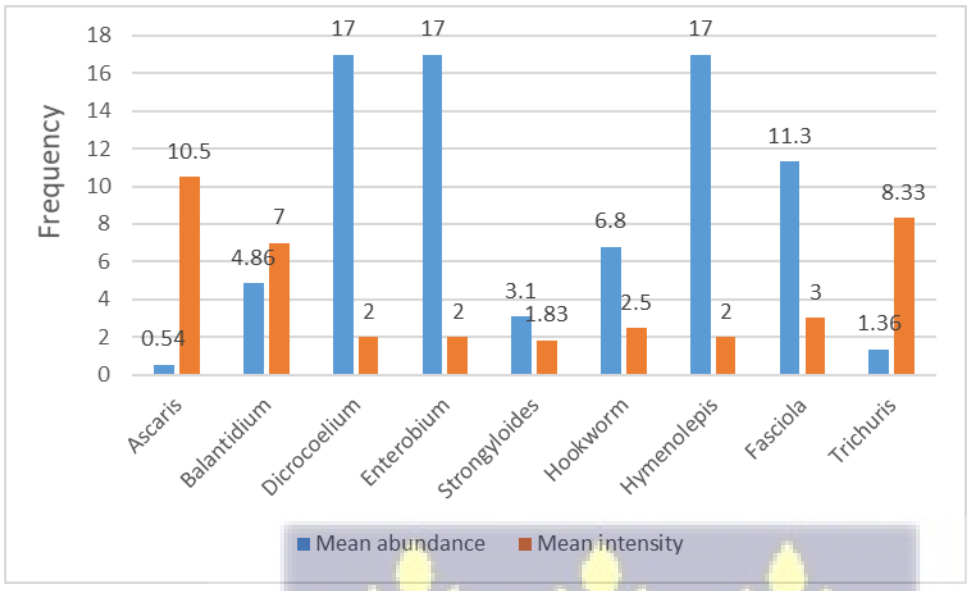

Figure 8: Mean abundance and Mean intensity of helminths identified in the faecal sample of small terrestrial mammals in Muni-Pomadze

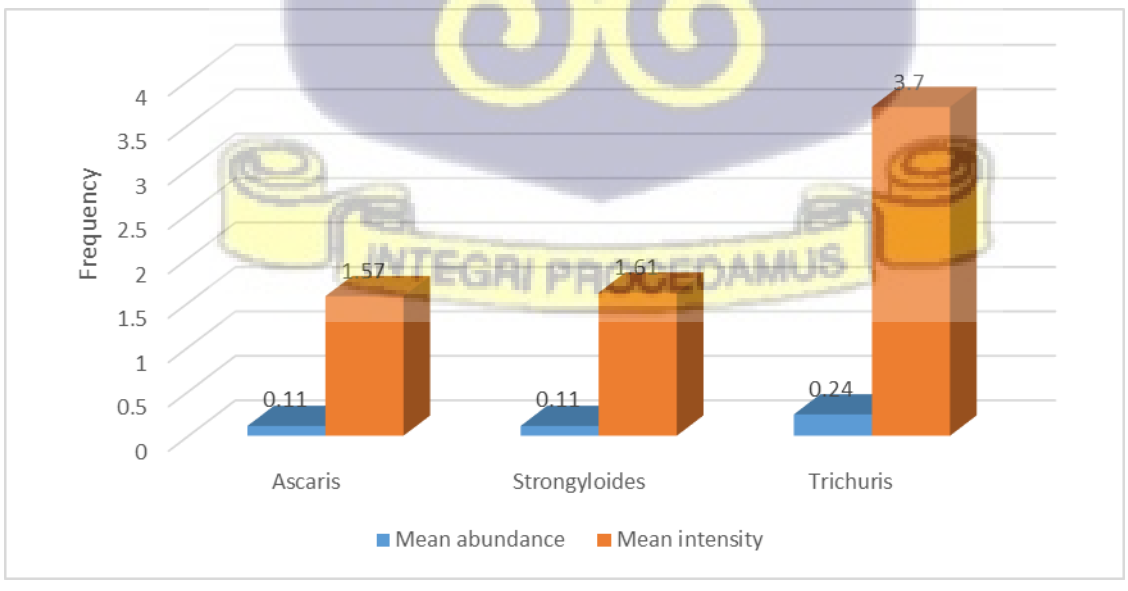

Figure 9: Mean abundance and Mean intensity of helminths identified in the faecal sample of small terrestrial mammals in The University of Ghana main campus

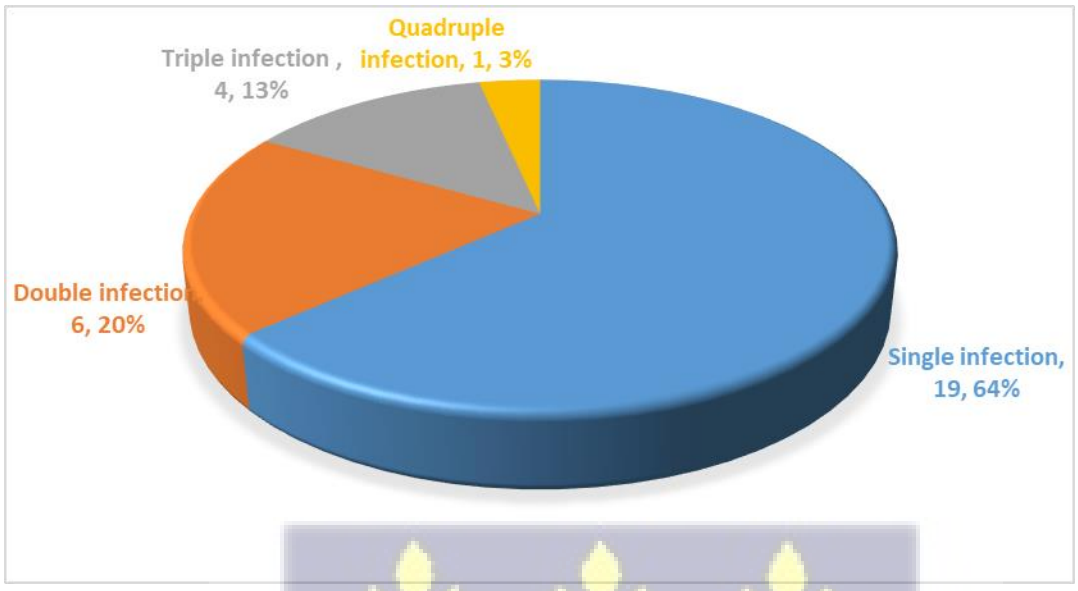

Figure 10. Parasite infection rate of small mammals in Muni-Pomadze ramsar site

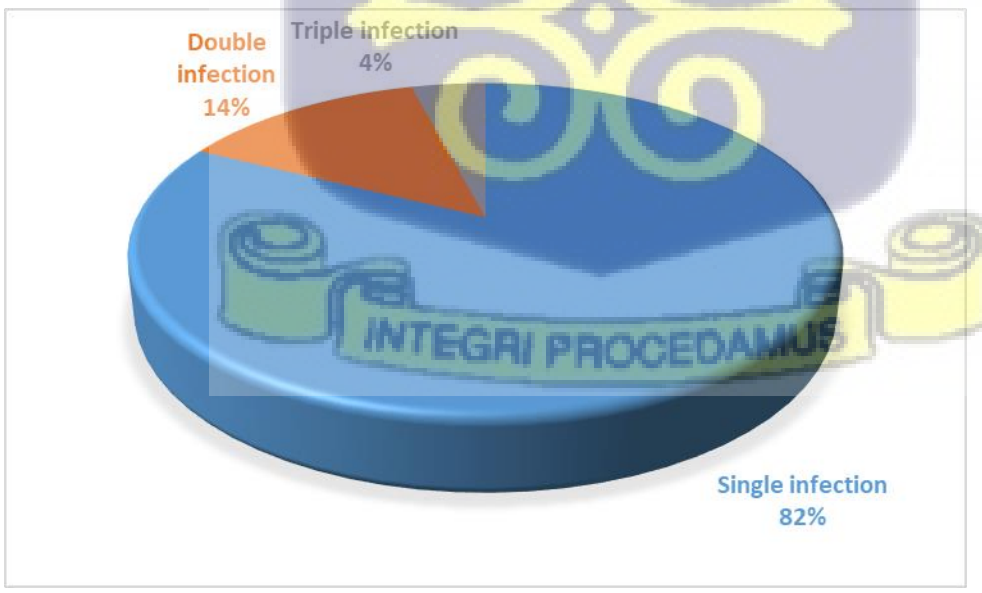

Figure 11. Parasite infection rate of small mammals in the grassland in the University of Ghana, Legon main campus

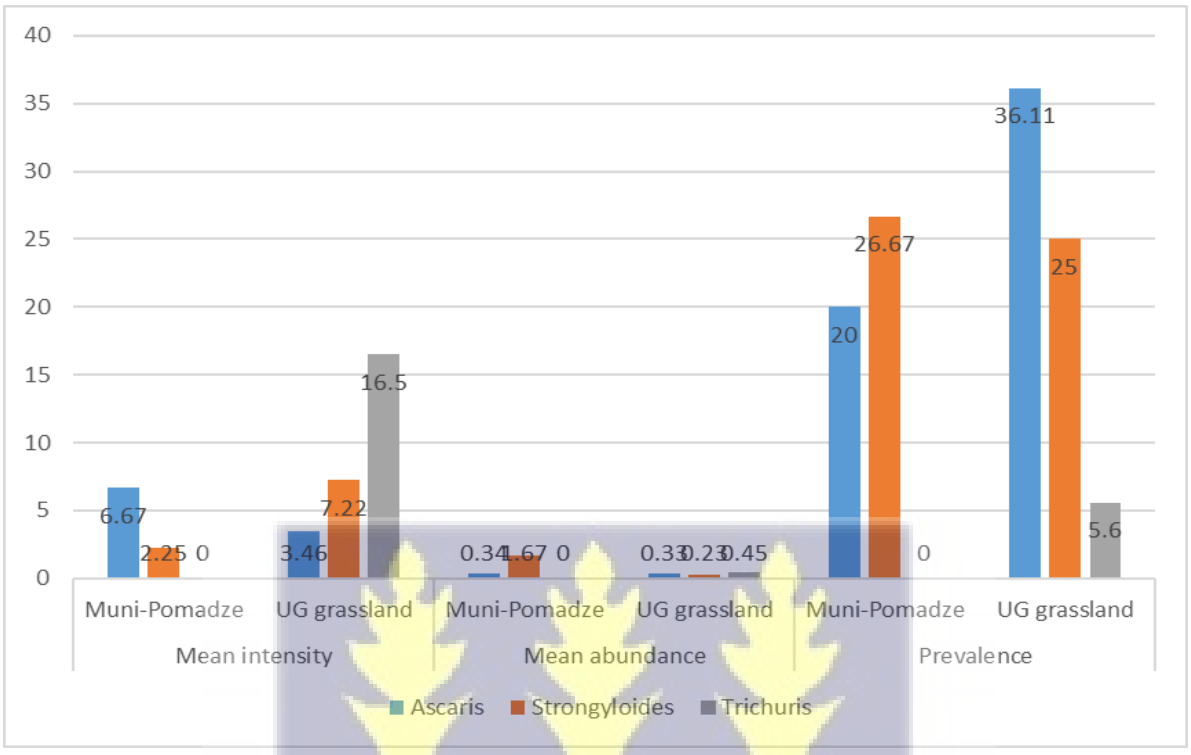

Figure 12: Mean intensity, mean abundance and prevalence of male small mammals from both study sites

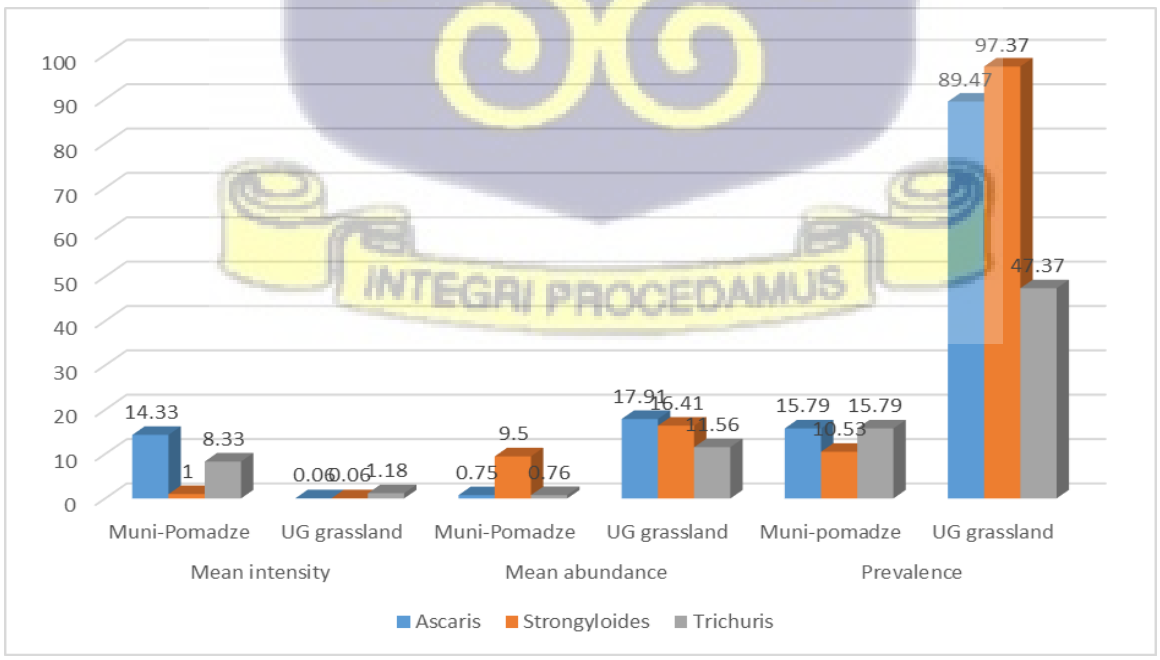

Figure 13: Mean intensity, mean abundance and prevalence of female small mammals

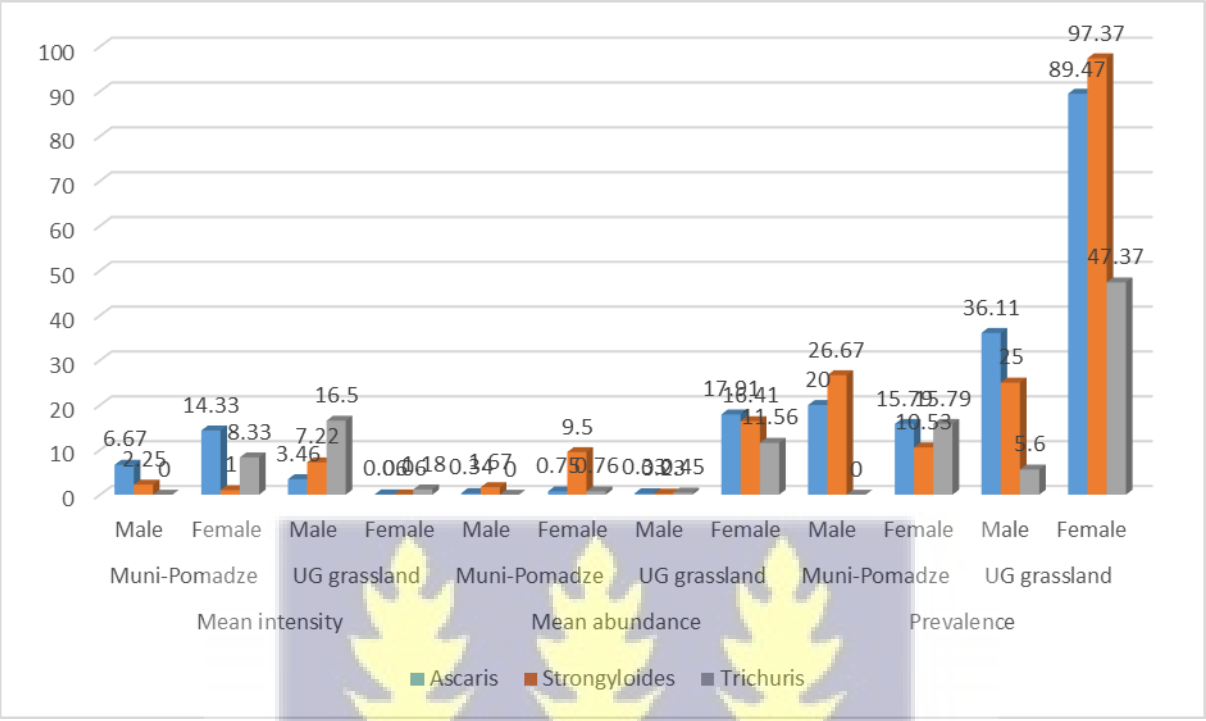

Figure 14: Mean intensity, mean abundance and prevalence rate of intestinal parasites in male and female small mammals from both study sites

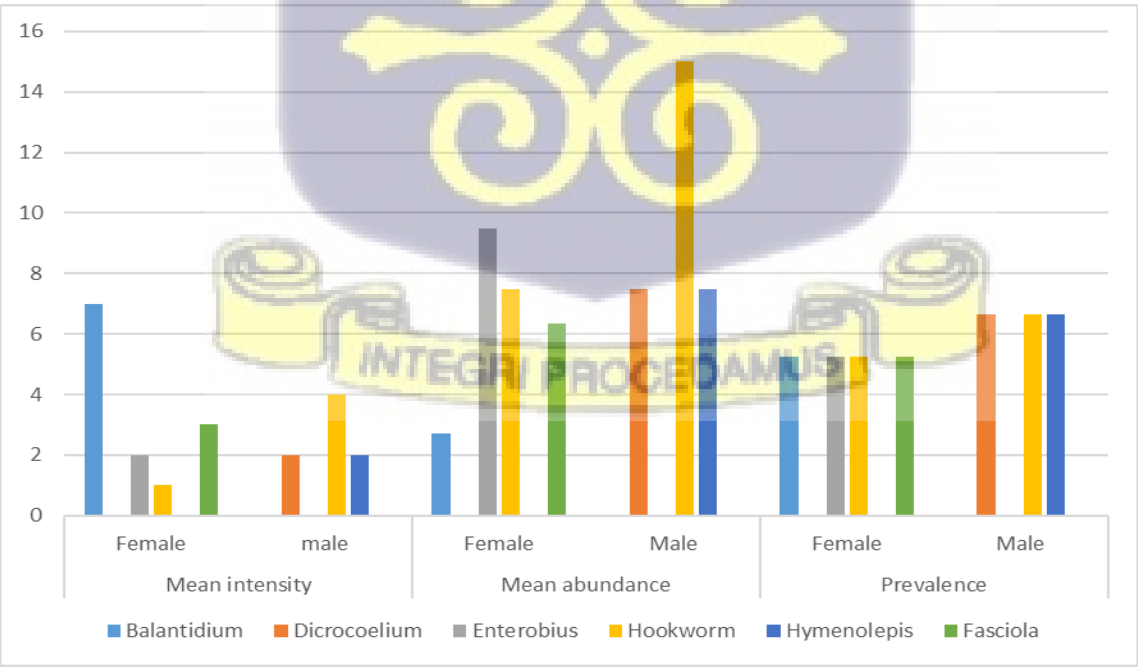

Figure 15: Mean intensity, mean abundance and prevalence of intestinal parasites identified in only small mammals from Muni-Pomadze ramsar site

4.3 Ectoparasites identified in the small mammals

Out of the two hundred and seventy-two ectoparasites identified, two hundred and sixty-six were nymphs and the rest were young adults of the species *Haemaphylasis leachi* (Fig. 11). Adults were dark brown with four pairs of legs whiles nymphs were pale in colour (Plate 4).

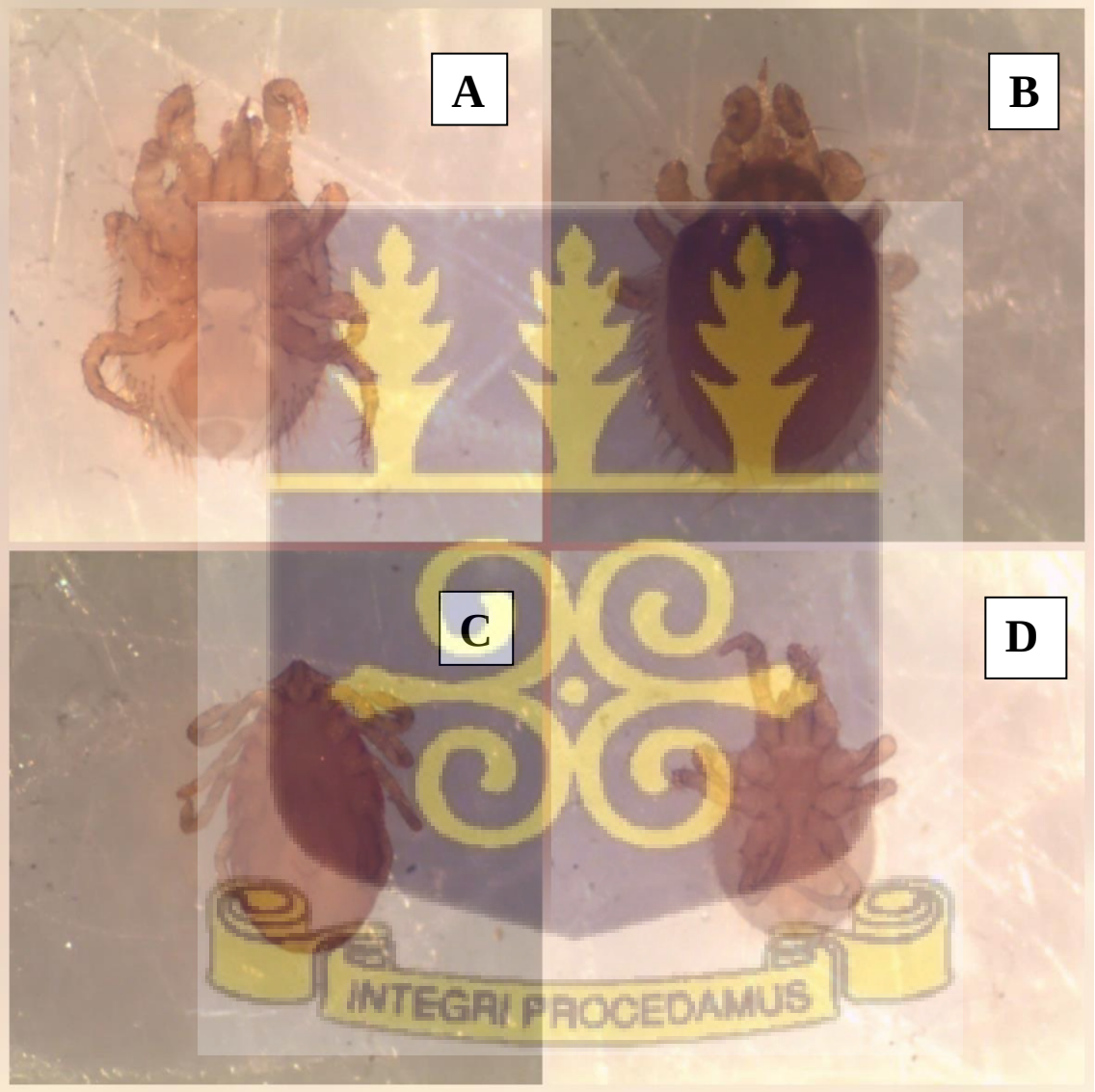

Plate 5. Pictures of *Haemaphysalis laechi*: A: Ventral view of adult B: Dorsal view of adult, C: Dorsal view of nymph, D: Ventral view of nymph

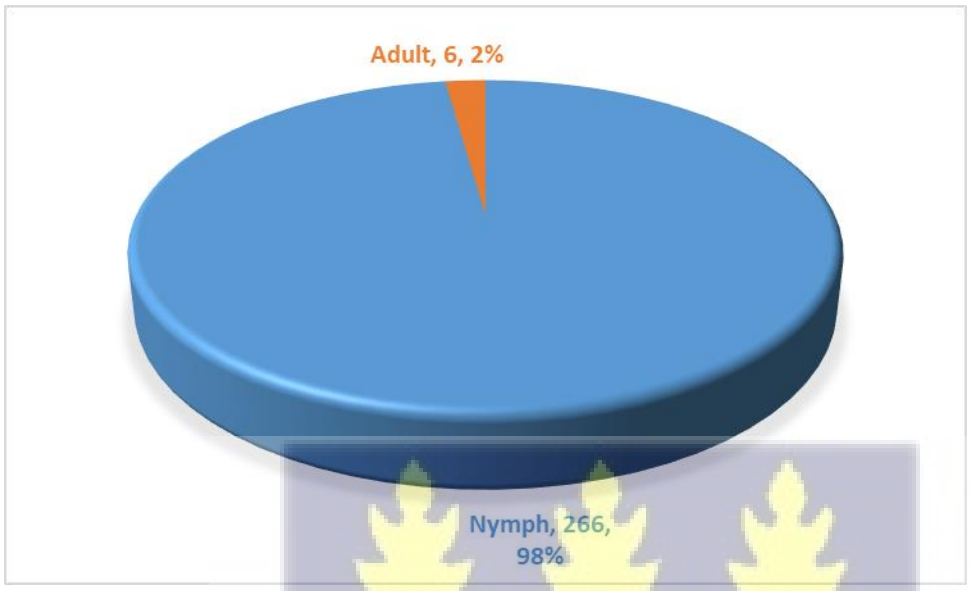

Figure 16. Status of ectoparasites identified from the captured small terrestrial mammals

CHAPTER FIVE

DISCUSSION

A total of nine species of intestinal parasites were identified including one Cestode, Nematode, Trematode and one Protozoa. The Cestode is *Hymenolepis* sp, the Nematodes are *Ascaris* sp., *Enterobius* sp., hookworm, *Strongyloides* and *Trichuris*, the Trematodes are *Dicrocoelium* and *Fasciola*, and the Protozoa *Balantidium*. This finding generally supports the intestinal parasites that are normally found in small mammals (Waugh *et al*., 2006; Coomansingh *et al*., 2009; Gudissa *et al*.; 2011; Adaeza *et al*.; 2017; Archer *et al*., 2017; Panti-May *et al*., 2013.)

Attuquayefio and Ryan, (1997) captured five species of rodents: *Lemniscomys barbarous, Mastomys erythroleucus Tatera kempi, Uranomys ruddi, Lemniscomys striatus,* but in this study only two species of small mammals – zebra mice (*Lemniscomys striatus*) and multimammate mice (*Mastomys natalensis*) were captured of which 85.3% were *Mastomys natalensis*. The difference in these two results could be due to several reasons: reduction in the biodiversity richness of Muni-Pomadze Ramsar site, difference in the season(s) during which the capture was made, methods of capture, baits used and differences in the duration of the two studies.

The medium through which parasites are transferred from wildlife to humans may occur through diverse ways, including through an infested wildlife host like the pelage of mammals (Wolfe and Wright, 2004). This study is similar to a study by Win *et al*., (2020) in the Central part of Myanmar, where a low infection rate with cestodes and nematode infection was recorded in small ruminants. The observed high *Ascaris* and *Strongyloides* prevalence underscores the importance of these small mammal infections as a potential zoonotic infection. Ideally, samples should have been collected from inhabitants that live close to the

study site so as to ascertain if these parasites of small mammals could be found in humans. However, this could not happen due to logistical constraints as well as the limited time and also the scope of this study.

The prevalence of *Ascaris* and *Strongyloides* in could be due to the presence of conditions that enhanced the growth of this helminth in the site. The low prevalence of 0.9% recorded for *Hymenolepis* spp. in this study is in sharp contrast to a study by Adaeza *et al*.; (2017), who recorded a high prevalence of 81.5% in Nsukka, a suburb of Nigeria, and Gudissa *et al*.; (2011), in Addis Ababa, and Pulscher *et al*, (2018); who identified *Dermacentor* spp. as the only tick species on small mammals they captured. This could be attributed to the small sample size of thirty-four small mammals. The tick species that is most prevalent on most small mammals is the *Haemaphysalis* sp (Cohan, 2019). The hundred percent prevalence of *Haemaphylasis leachi* as ectoparasites on the small mammals could be due to the relatively small size of the host and hence the corresponding size of the ectoparasites also had to be small.

This study is in contrast to the report by Raharivololona and Ganzhorn in 2007, where all small mammal species captured had high prevalence infection rates (> 73%) and were hosts from three to more than twenty gastrointestinal parasite forms. Another study in Nigeria demonstrates a high prevalence (83%) of blood parasites in small terrestrial mammals (Ajavi *et al*., 2006). Akinboade *et al*, 1981); conducted a research in Edo state, and reported 17 helminth species in eight species of small mammals. Variations in parasite prevalence among studies may be related to different sample size, geographical features, the season in which sampling was done, and diagnosis methods used (Morand, 2015; Barelli *et al*., 2021). The overall prevalence of helminths in commensal rodents is influenced by different environmental conditions (Panti-May *et al*., 2013). Panti-may reported 77% of rodents

captured in Mexico were infected with gastro-intestinal helminths which is higher than what was observed in this study

The low prevalence of helminth in this study could be due at least two months interval between specimen collection and laboratory analysis, and the use of only modified Zinc Sulfate flotation technique. The roundworm *Ascaris* sp, probably *Ascaris lumbricoides*, is one of the helminth eggs of public health importance that did not infect rodents, but were mechanically transported by the rodents (Archer *et al*., 2017).

There was no significance difference between helminth infection and sex in Muni-Pomadze Ransar Site since all $p>0.05$ except in *Trichuris* ($p=0.0251$), where it was more common in female captured small mammals than males. There was no significance difference between helminth infection sexing males and females in UG main campus except for *Strongyloides*($p<0.001$) where it was more common in female than males.

The infection rate in Muni-Pomadze was 32.4% which is similar to a study by Singla *et al*. where the infection rate of rodents was 35.2%. In the present study, 64% of small mammals from Muni-Pomadze had a single infection whiles 36% had multiple infection. In the UG main campus, 82% had a s ingle infection while 18% had multiple infection. This is similar to studies by Panti-May who reported 81.8% of rodents with single infection, and also Singla *et al*. who reported a mixed infection of helminths including Cestodes and Nematodes

The differences in infection rates may be due to particular characteristics of habitat, the helminth species present in the area, as well as the behaviour and relative abundance of the hosts (Panti-May *et al*., 2013). The high mean abundance and mean intensity of the grassland in the University of Ghana shows that individual small mammals had more eggs per individual than in Muni-Pomadze.

This study identified nine species of gastro-intestinal parasites which is similar to Coomansingh-Springer *et al.* where they identified ten species of helminths. Similar surveys in Jamaica and Grenada revealed 9 and 6 helminths respectively (Waugh *et al.*, 2006; Coomansingh *et al.*, 2009). This findings of single infections being more common than mixed infections is similar to previous studies conducted in Jamaica (Waugh *et al.*, 2006) and Grenada (Coomansingh *et al.*, 2009) where single infections were more common than mixed infections.

Considering the role of rodents in the spread of zoonotic parasitic agents in the environment and their risk for public health and the high infectivity rates of zoonotic helminthic species in rodents, it seems a comprehensive control plan and a preventive approach are required (Mohtasebi *et al.*, 2020).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The small terrestrial mammals captured in this study were *Mastomys natalensis* and *Lemniscomys striatus*. In Muni-Pomadze: 4 males and a female of *L. striatus;* 18 females and 11 males of *M. natalensis*. In The University of Ghana grassland, 36 males and 38 females of *M. natalensis*. Gastrointestinal parasites identified were *Ascaris* sp., *Balantidium, Dicrocoelium* sp., *Enterobius* sp., *Fasciola* sp., *Hymenolepis* sp., *Hookworm*, *Strongyloides* sp., *Trichuris sp*. *Ascaris sp was was the dominant species*. XXXX Ectoparsites were of one species – *Haemaphysalis leachi*, most of which were nymph.

The limitations of the study include the inability to carry out the molecular work due to lack of funds even though blood samples were collected onto filter paper for that purpose. The zoonotic aspects of the study could not also be established as the molecular work was not doen, neither could humans be included in the study. Ideally, samples should have been collected from inhabitants that live clode to the study site so as to ascertain if these parasites of small mammals could be found in humans. However, this could not happen due to logistical constraints as well as limited time and also the scope of study.

6.2 RECOMMENDATION

Even though the original plan of the study was to include molecular work, this aspect could not be done due to the lack of funds. Future studies should characterize the parasites at the gene level to the species level. Future studies should also consider collecting samples in the dry and rainy seasons in order to get a complete overview of the small mammals and their parasites. Future studies should also include human samples to establish the zoonotic implications of such observations in the small mammals.

The samples were collected in November and the laboratory analysis was done in March due restrictions from the COVID-19 pandemic. It is recommended that in the future the laboratory work should not take more than a week after sample collection in order to reduce the chances of worms, larvae, eggs etc of parasites becoming disfigured.

Using morphology alone for identification may introduce wrong names where species are closely related. Trying to differentiate the parasites based on morphological and metrics may be difficult and hence molecular work should also be included in further studies. Blood samples should be taken from inhabitants close to the Muni-Pomadze Ramsar site to ascertain the public health importance of the parasites found in this study.

Further research will be required to properly investigate these findings.

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LIST OF APPENDICES

APPENDIX I

Equipments and reagents

- Cotton wool
- Blood lancet
- Slide rack
- Microscope slides
- Light microscope with x40 and x100(oil) objective lens (Leica microscope)
- Grease pencil
- Disposal gloves
- 10% formalin
- Methanol
- Ethanol
- GPS

APPENDIX II

PREPARATION OF REAGENTS

Physiological saline

8.5g of saline was dissolved in a liter of distilled water.

Zinc Sulphate solution

386g of ZnSO4 was dissolved in a liter of distilled water and warmed to make dissolution faster.

APPENDIX III

Grassland in the UG main campus

Oneway Analysis of Ascaris sp By SEX

Kruskal-Wallis Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
3.6909	1	0.0547

Oneway Analysis of Strongyloides By SEX

Kruskal-Wallis Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
16.1023	1	<.0001*

Oneway Analysis of Trichuris By SEX

Kruskal-Wallis Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
3.3685	1	0.0665

Muni-Pomadze ramsar site

Oneway Analysis of *Ascaris* sp By Sex

ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
0.1095	1	0.7407

Oneway Analysis of *Strongyloides* sp By Sex

ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
0.9660	1	0.3257

Oneway Analysis of *Balantidium* By Sex

ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
0.9660	1	0.3257

Oneway Analysis of *Fasciola* sp By Sex

ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
1.3103	1	0.2523

Oneway Analysis of *Hymenolepis* sp By Sex

ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
4.2236	1	0.0399*

Oneway Analysis of Hookworm By Sex

ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
3.4643	1	0.0627

Oneway Analysis of *Trichuris* sp By Sex
ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq

ChiSquare	DF	Prob>ChiSq
4.6624	1	0.0308*

Oneway Analysis of *Dicrocoelium* sp By Sex

ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
4.1628	1	0.0413*

Oneway Analysis of *Enterobius* sp By Sex

ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
0.9804	1	0.3221