COMMON PARASITES OF FRUIT-EATING BATS IN SOUTHERN GHANA

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BY

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DECLARATION

This is to certify that this thesis is the result of research undertaken by me, Naa Awula Narkie Nartey towards the award of an MPhil degree in Zoology (Parasitology option) at the Department of Animal Biology and Conservation Science, University of Ghana. This thesis has not been submitted either in part or in whole for any other degree and all reference to other people's work has been duly acknowledged.

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DEDICATION

This work is dedicated to God Almighty, my supportive family who have been of great help in every aspect of the work and also to my good friends Evans Ferguson, Jones Quartey and Kofi Amponsah-Mensah who have also helped in diverse ways for the completion of this thesis.

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LIST OF ABBREVIATIONS

AIDS- Acquired Immuno-Deficiency Syndrome

AMA- Accra Metropolitan Assembly

CDC – Centre for Disease Control and Prevention

DNA - Deoxyribonucleic Acid

EID – Emerging Infectious Disease

FAO - Food and Agricultural Organization of the United Nations

GPS - Ghana Population Census

HeV- Hendra Virus

LED - Light-emitting diode

NiV- Nipah Virus

PBS - Phosphate buffered saline

PCR - Polymerase Chain Reaction

RNA- Ribonucleic Acid

SARS - Severe Acute Respiratory Syndrome

SDSS - Sustainable Development Success Stories

TAE - Tris base, acetic acid and EDTA

USA - United States of America

UV - Ultraviolet light

WHO - World Health Organization

WNS – White Nose Syndrome

ABSTRACT

Bats are nocturnal mammals found everywhere except in the Antarctica and Arctic regions. They are important for the maintenance of the ecosystem in terms of pollination and seed dispersal, particularly in tropical regions, as well as in controlling insect populations that would otherwise be pests. Bats have been reported as reservoir hosts for several pathogens including viruses such as Ebola, Nipah, Hendra and Lyssa. The purpose of the study was to document the parasites that infest and infect bats and to determine their potential public health importance to humans. Four hundred and eighty bats were captured, using mist nets, at four different locations in Ghana ("37" Military Hospital, Buoyem, Tanoboase Sacred Grove and Ve-Golokuati) from March to August 2014. Ectoparasites were collected through hand picking and also by swabbing with cotton wool soaked in 70% ethanol. Haemoparasites were studied by taking a drop of blood (0.05ml) from the propatagial vein of each bat and a blood smear prepared. Drops of blood were also put on filter paper for molecular work. Faecal samples were taken for examination for helminth parasites. Of the total number of bats examined, 159 (33.13%) were infested with ectoparasites. Four species of bat flies, eighteen species of mites and two species of ticks were recorded. The most abundant ectoparasite group found on bats were mites; where the species most frequently encountered were *Spinturnix americana*, *Carpoglyphus* sp. and *Ancystropus zeleborii*. *Nycteribia alternata* and *Cyclopodia greefi greefi* were the most common bat flies recorded while *Argas vespertilionis* was the most common tick found to infest bats. Adult bats ([104 out of 184]56.52%) harboured the most ectoparasites compared to sub-adult (46.90%) and juvenile (29.53%) bats. Male bats also harboured significantly higher numbers of ectoparasites than female bats (p= 0.01584). With respect to capture site, the highest proportion of individuals

infested was recorded at the Tanoboase Sacred Grove (50%). Bats examined at the '37' Military Hospital were the least infested (20.83%). The highest proportion of bats infested was recorded in March (44.06%) and the least in August (20%). Haemoparasites were observed in the blood samples with almost half of the bats (~48%) infested. The specific parasite species, however, could not be determined due to logistic and time constraints. From the faecal examination, none of the bats captured were infested with helminths. Eleven of the parasite species recorded on bats in this study are known to transmit several pathogens. For example ticks transmit *Borrelia* sp., *Rickettsiae* sp. and *Ehrlichia* sp.; mites transmit enteric acariasis, scrub typhus and *rickettsial* pox and bat bugs transmit trypanosomes.

Given the important roles bats play in the ecosystem, their role in the transmission of zoonotic diseases and the vast range of parasites that they harbour, further studies would be required to understand the bat-parasite-disease dynamics. This knowledge is necessary to inform bat conservation interventions so decisions taken take into consideration public health issues.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Bats are nocturnal mammals that are important for the maintenance of the ecosystem. Bats are the only flying mammals; they are found everywhere except in the Antarctica and Arctic regions (Airas, 2003) and perhaps are the most abundant, diverse and geographically dispersed vertebrates. Although bats are relatively common in temperate regions, they reach their greatest diversity in tropical forests (Hill and Smith, 1984; Vaughan *et al*., 2000). Due to the fact that bats are highly mobile and move over considerable distances, they are ideal dispersers of not only seeds but also of diseases.

As mammals, bats are viviparous; they belong to the second largest mammalian order (Chiroptera) with 966 species outnumbered only by rodents with about 1700 species (Altringham, 1996). Globally, 1232 species of bats have been described (Schnipper *et al*., 2008). Eighty four species of bats have been recorded in Ghana (Grubb *et al*., 1998).

Bats range in size from the smallest, the bumblebee bat (*Craseonycteris thonglongyai*) which weighs 1.5-2g to 1kg flying foxes (*Pteropus* spp.) with wingspan approaching two metres (Airas, 2003). The largest bats attain a wingspan of 1.7 metres weighing up to 1.6 kg (Nowak, 1991). The wings of bats are modified forelimbs with the surface covered with skin and supported by four fingers. The flight membrane usually extends down the sides of the body and attaches to the hind limbs. Bats also have a tail membrane called the uropatagium. The hind limbs in particular are generally short and small with sharp, curved claws that help bats cling to surfaces in their roost (Hill and Smith, 1984; Vaughan *et al*., 2000). Bats in general have specific roosting requirements which differ among species. They may roost in

caves, crevices, trees, under logs and even in human dwellings.

1.2 Bat biology

Bats belong to the order Chiroptera (meaning hand wing) which has two sub-orders, the megachiroptera also known as the megabats and microchiroptera also known as the microbats. All of the megabats belong to the same family, Pteropodidae (the old world fruit bats) while the microbats belong to four superfamilies with a total of 17 families (Altringham, 1996).

1.2.1 The Megachiropterans

The megachiropterans, also known as the Old World bats, are large and live in the tropics. They feed mainly on fruits, flowers, pollen and nectar. Megachiropterans have a good sense of smell which they use to locate ripe fruits during their hunting at night. They also rely on vision to orient in the dark at night and thus have large prominent eyes. Most species of fruit bats cannot echolocate and roost mainly in trees and shrubs, except the Egyptian fruit bat (*Rousettus aegyptiacus*) which are able to find their way by echolocation (Rosevear, 1965). The megachiropterans, contrary to the name, are not always large, the smallest species in this group is about 6cm long and thus smaller than some microchiropterans. However, megachiropterans are generally larger than the microchiropterans, with forearm lengths of 40-220mm, weight from 20g to 1.5kg and a wing span approaching 2m. They are found in sub-Saharan Africa, South Asia and Western Oceania, including northern Australia (Airas, 2003). These bats have dog-like faces and are well equipped with sharp teeth and powerful jaws to be able to break tough skinned fruits. Most megachiropterans are brown in colour.

Megachiropteran species control their body temperature within a tight range of temperatures and none hibernates (Nowak, 1991; Vaughan *et al*., 2000).

1.2.2 The Microchiropterans

Microchiropterans are generally smaller than the megachiropterans (4 to 16 cm long) (Whitaker *et al*., 2004) with forearm length ranging from 22 to 110 mm (Airas, 2003). While all microchiropteran families prey upon insects to some extent, a minority have evolved to feed on vertebrates, blood, fruit, nectar and pollen, (Altringham, 1996). Microchiropterans have the ability to use echolocation during hunting and to avoid obstacle. Microchiropterans are either tree hollow roosting or cave roosting bats. They inhabit a wide range of habitats with vegetation cover ranging from wet and dry forests, swamps, rain forests to open farm lands and suburban areas (Rosevear, 1965). Microchiropterans, unlike megachiropterans, have labile body temperatures and some hibernate (Hill and Smith, 1984; Nowak, 1991; Vaughan *et al*., 2000).

1.3 Parasite infestation

Like all other animals, bats are associated with internal and external parasites (Hill and Smith, 1984). A parasite is any organism that derives benefit from living in or on another organism (the host) at a cost to the host (Northrop-Clewes and Shaw, 2000). Parasites are classified into ectoparasites and endoparasites.

1.3.1 Ectoparasites

Ectoparasites are organisms which inhabit the skin or outgrowths of the skin of another organism (the host) for various periods, and may be detrimental to the host (Hopla *et al*., 1994). They live in the fur, wing membranes and other parts of the host's body where they feed on host's blood. Most of them have flattened bodies for easy movement through the fur of their hosts and sucking mouthparts for sucking blood from their hosts. Bats commonly harbor external arthropod parasites such as ticks, mites, bugs and fleas. The parasite species that infest bats exhibit a range of host specificity, some are found on one or a few species; while some occur on a wide variety of bat species (Hill and Smith, 1984).

1.3.2 Endoparasites

Endoparasites are parasites that live in the internal organs or tissues of its host. Bats are known to harbor several protozoans that cause malaria (eg Plasmodium, Hepatocystis, Nycteria and Polychromophilus) (Schaer *et al*., 2013) as well as intestinal parasites. Trypanosome protozoans (*Trypanosoma cruzi* and *Trypanosoma rangeli*, Thomas *et al*., 2007; *Trypanosoma dionisii, Trypanosoma vespertilionis* and *Trypanosoma incertum*, Molyneux, 1991) that may cause a variety of diseases such as sleeping sickness have been recorded in a number of bat species. Many flatworms (Cestoda and Trematoda) *Posthodendrium panouterus* and *Hymenolepsis kerivoulae* (Okafor *et al*., 2004) and roundworms (Nematoda) *Rictularia chaeraphoni* and *Histostrongylus coronatus* (Okafor *et al*., 2004) spend at least part of their life cycle within the tissues of bat hosts (Wund and Myers, 2005).

1.4 Justification

Myths surrounding bats are so many that it is ludicrous to believe that these important animals are really responsible for all the evil they are associated with. In many cultures, bats are symbols of ghosts, death, diseases and backwardness. For example in the Ve-Golokuati township of the Volta region of Ghana, the inhabitants believe that the inability of the town to progress is mainly because bats hung upside down when resting and therefore the progress of the people is also turned upside down. Some cultures in Africa believe bats can induce madness (Johannesburg Zoo, 2014) and many paintings from Western Europe in the middle ages depict the devil with bat wings (Ramel, 2014). In contrast, there are also legends that associate bats with good fortune, for instance bats symbolize long and happy life in many Chinese cultures (Knowles, 2009). Ancient Egyptians also believed that bats could prevent or cure poor eyesight, toothache, fever and baldness and that if hung over the doorway of a home, could prevent entry of demons that carried these 'diseases' (Kunz, 1984).

Bats are important in pollination and seed dispersal, particularly in tropical regions. Many rainforest trees depend on bats for pollination and seed dispersal which is particularly important in facilitating regrowth after forest clearance (Knowles, 2009). Similarly many tropical plants depend on the activities of bats for the distribution of their seeds. It is estimated that there are some 300 bat-dependent valuable plant products, including chewing gum, tequila, sisal, medicines, dyes and fuel (Knowles, 2009). Guano which is bat droppings is used as fertilizer. Some bats are keystone species on which the health of whole ecosystems depend. Insectivorous bats significantly reduce insect populations that would otherwise be pests. There is therefore the need to conserve these animals as they are the major species on which whole ecosystems depend on for their sustainability.

Bats can transmit various diseases to human beings. Indeed, they serve as reservoir hosts for a long list of pathogens including viruses such as Ebola, Nipah, Hendra and Lyssa (Calisher *et al*., 2006). The Severe Acute Respiratory Syndrome (SARS) outbreak in 2002 in Asia and the transmission of Middle East Respiratory Syndrome (MERS) to humans in the Middle East in 2013 could be traced back to viruses that have switched hosts from bats to humans (Schaer *et al.*, 2013). Bats have an exceptional immune system that can hold in check many pathogens which are usually deadly in humans (Schaer *et al*., 2013).

In recent years, populations of bats have declined drastically in some regions of the world and many more species are now endangered (Mickleburgh *et al*., 2002). This has come about due to destruction of bat habitat, cave exploration and the use of pesticides, among many others. Bats also continue to decline as they are a delicacy for some people especially in the tropics (Kamins *et al*., 2011). In some cases, bats have been destroyed because they have been perceived as pests due the loud honking noises they make. The decline in bat populations has not only been caused by activities of humans but also by diseases that plague them (Lametti, 2011). Although they have a high tolerance for diseases, bats can also be affected by diseases and suffer the symptoms and eventually die. For instance, in the temperate regions, a lot of bats are unable to recover from hibernation during the winter period due to the White Nose Syndrome (WNS), a fungal disease. This disease has killed millions of bats in the United States of America and is capable of wiping an entire hibernating population. Some temperate bats have been encountered in the tropics (Hamilton *et al.*, 2012) and therefore likely that this disease will begin to occur in the tropics. Due to the destruction of bat habitat, they are forced to live closer to humans, livestock and pets than they naturally would. *Epomophorus gambianus* for instance have been reported to

move to urban areas due to encroachment by humans into its natural habitat. Bats may even feed on fruits in the backyard of homes and as they do so, leave their saliva, urine and faeces on fruits, feeding sites and household furniture which poses risk of disease transmission to other animals and humans. Not only do bats transmit diseases by their foraging behaviour but also through the parasites that they are infested with. Parasites serve as vectors for the transmission of diseases to other bats and humans. Bat parasites and the pathogens they harbour (for eg. *Ricketssia* sp. in *Argas persicus* from Ethiopia; Pader *et al.,* 2012; *Bartonella* sp. in bat flies from Nigeria; Kamani *et al*., 2014) have been extensively studied in some countries. A few of such studies have been carried out in Ghana (Billeter *et al*., 2012) but knowledge is still lacking especially with respect to zoonotic diseases that the parasites are capable of transmitting. Knowledge of the range of parasites that infest bats is required for better understanding of parasitic diseases in bats as well as human-bat interactions and potential for spillover of zoonotic diseases from bats to humans and domestic animals.

1.5 Objectives

1.5.1 Overall objective

This study aims at investigating external and internal parasites harbored by bats and their potential health risk to urban and rural dwellers in Ghana.

1.5.2 Specific objectives

The specific objectives are to:

- To identify the range of parasites that are harboured by bats in four study sites ('37' Military Hospital, Buoyem, Ve-Golokuati and Tanoboase Sacred Grove).

- To determine the effects of bat bionomics on the prevalence and intensity of parasite infestations and infections.
- To investigate the potential role of bats as reservoirs of zoonotic diseases.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Emerging Infectious Diseases

Infectious diseases continue to emerge and the majority of these are zoonotic in origin (Woolhouse and Gowtage-Sequeria, 2005; Jones *et al*., 2008). Emerging infectious diseases are defined as those that have recently increased in incidence or geographic range, recently infected a new host population, recently been discovered or are caused by a newly emerged pathogen (Daszak *et al*., 2001). Zoonotic infections originating in wildlife are a significant threat to human health (Daszak *et al.*, 2000) and have for many years contributed to the cause of death and disability worldwide. They do not only cause deaths and disabilities but also cause economic instability and global unrest. An example is the Ebola virus outbreak in West Africa. This represents the first and largest outbreak of the viral disease in this part of Africa affecting multiple countries (Meredith *et al.*, 2014). Although this virus has been infecting humans since 1976, its sporadic occurrences had caused no emergency intervention until now. As of mid-September of the year 2014, 5,843 suspected cases had been reported resulting in the deaths of 2,803 (WHO, 2014a) and causing fear and panic not only in the affected nations but also worldwide. Disbursement of several millions of dollars, which could be used for other developmental projects, has been given by the World Health Organization to affected nations to control the spread of the disease. Fruit bats are suspected to be the wildlife reservoir of Ebola.

Zoonotic infections, particularly those caused by RNA viruses, have been recognized as a significant human health threat, particularly in developing countries (Maudlin *et al*., 2009) where health systems are week and not very effective as in the developed countries. There

are five major types of infectious agents that affect humans and animals which are bacteria, viruses, fungi, protozoa, and helminths. These infections account for at least twelve percent of all human pathogens (Taylor *et al*., 2001).

Some emerging infectious diseases include *Bartonella* sp., *Borrelia* sp., Acquired Immuno-Deficiency Syndrome (AIDS), Severe Acute Respiratory Syndrome (SARS) coronavirus, Nipah virus and the Ebola virus. The past decades have seen a dramatic resurgence of zoonotic diseases that affect only wild animals, spill over into human populations. This has been attributed partly to climate change and the destruction of wild habitats (Bitam *et al*., 2010) thereby causing the emergence of new diseases and the infiltration of pathogens into new areas which have previously not encountered these diseases. This has thwarted the efforts made in the previous years to control disease outbreaks in humans. Bats are host to a range of zoonotic and potentially zoonotic pathogens.

Despite the rate at which zoonotic infections spread very easily and quickly, the dynamics of how this happens is not well understood. In the case of the Ebola virus outbreaks, over the years, several suggestions have been made on how this virus is able to enter human populations. An isolated case in Ivory Coast in 1994 occurred following autopsy of an infected chimpanzee carcass in the forest (Georges *et al*., 1999). Other outbreaks between 2001 and 2003 occurred after hunters had handled animal carcasses (mainly gorillas, chimpanzees and duikers) found in the forest (Leroy *et al*., 2004). In this case, Leroy *et al*. (2004) were able to reconstruct the likely initial human-human transmission events that preceded the outbreak and found out that the first contact was a human victim who had bought freshly killed bats from hunters to consume. This demonstrates how the disease-

causing pathogen can move freely from one host to another and in this case, from bats to humans. Many emerging infectious diseases spring up so fast that it is difficult to keep up with them in terms of vaccine development and treatment. They spread so fast that there is barely time to understand the etiology of the diseases to be able to keep them under control. Vaccines and treatments are therefore not readily available for some of these diseases.

2.2 Bats as reservoirs of zoonotic diseases

Bats have been implicated in the emergence of many high profile zoonotic diseases of public health importance including rabies, Ebola, Nipah, Hendra and Severe Acute Respiratory Syndrome (SARS) related coronaviruses (Calisher *et al*., 2006). Their relevance to human health has increased the interest in bats as potential reservoir hosts and vectors of zoonotic pathogens (Mühldorfer, 2013) since the 1940's, and is thought to have peaked in the 1980's (Jones *et al*., 2008). The high mobility of bats, broad distribution, social behaviour (communal roosting, fission–fusion social structure) and longevity also make them ideal reservoir hosts and sources of infection for various etiologic agents (Bai *et al*., 2011).

Bats harbour deadly viruses, bacteria, protozoans and other disease-causing pathogens. For example Nipah virus (NiV), a deadly virus harboured by bats, caused significant human mortality (~40%) during its initial outbreak in Malaysia in 1998 (Chua *et al*., 2000) and has emerged repeatedly in Bangladesh and India since 2001 (Lo *et al*., 2012; WHO, 2013). Nipah virus also causes severe disease in animals such as pigs and other domestic animals as well as severe acute respiratory syndrome and fatal encephalitis in humans, although some people show no symptoms (WHO, 2014a). Henipavirus is an emerging zoonotic pathogens capable of causing illness and death in domestic animals and humans (Sawatsky *et al*., 2008).

Although Hendra Virus Diseases is rare, it could cause fatal respiratory or neurological disease in humans. Marburg virus disease is another rare hemorrhagic disease that affects humans and other primates causing serious illness with high mortality. The reservoir host of this virus is the African fruit bat, *Rousettus aegyptiacus*. However, they do not show any obvious signs of illness (Swanepoel *et al.*, 2007).

SARS, also harboured by fruit bats, is a serious form of pneumonia disease which causes acute respiratory distress, pulmonary fibrosis, osteoporosis and femoral necrosis in humans (Holmes, 2003). Bats have been known also to harbour rabies and this has caused several outbreaks in human populations (the Peruvian jungle, Lopez *et al.*, 1992; Northern Brazil, Da Rosa *et al.*, 2006). Rabies virus is transmitted between mammals, primarily through the inoculation of the rabies virus (during bites) present in the saliva of infected individuals (McKendrick, 1941). Outbreaks of rabies have been found not only in human populations but also in populations of Egyptian fruit bats (Kwiecinski and Griffiths, 1999). This virus has caused serious illness in these bats and often lead to their deaths (Klimpel and Mehlhorn, 2014). Other less known viruses that occur in fruit bats include Uganda S virus, Yogue virus, Kasokero virus (Calisher *et al.*, 2006); these have not been proven to be transmitted to humans and other animals as yet. Rift Valley fever virus and Toscana virus are other viruses harboured by bats that also affect humans.

In addition to viruses, bacteria and parasites that can potentially cause human infection (Gill *et al.*, 2004) have been detected in bats (Loftis *et al.*, 2005; Reeves *et al.*, 2007). Studies have confirmed the presence of *Bartonella* sp. in bats from Peru, Guatemala, Kenya, and the United Kingdom (Concannon *et al.*, 2005; Kosoy, 2010; Bai *et al.*, 2011). *Borrelia* sp.,

Ehrlichia sp. and *Rickettsia* sp. have also been found in bats. Due to the fact that some bat species share roosts, in which thousands of bats occupy, it creates the opportunity for the transmission of parasites as well as the infectious pathogens they harbour. Various ectoparasites have been documented to transmit pathogens to bats but the possibility of disease transmission to humans is not known (Estrada-Pena and Jongejan, 1999; Bitam *et al.*, 2010).

Bats themselves are not exempt from emerging pathogens. The devastating effects of White-nose syndrome in the United States of America highlights the fact that bats also suffer and die from emerging infections. The fungi (*Pseudogymnoascus destructans)* that grows on the muzzles and wings of wintering bats account for at least 5.7 million to 6.7 million deaths of North American bats (Froschauer and Coleman, 2012). About seven species have been documented to be affected by the fungus; *Eptesicus fuscus, Myotis lucifugus, Myotis septentrionalis, Perimyotis subflavus* (Blehert *et al.*, 2009); *Myotis leibii, Myotis grisescens* and *Myoti sodalis*. The fact that large numbers of bats die each year from this disease, is very alarming and a cause for worry since bats are important species to the maintenance of the ecosystem. This will however be very costly in the tropics where about 500 topical plant species are completely, or partially, dependent on bats for pollination (Heithaus *et al.*, 1974). Bats are the main pollinators of major tropical forest (African baobab tree, kapok and floss-silk tree) and fruit species (banana, mangoes, avocado and cashew) and also predators of nocturnal insects which may otherwise be pests (Boyles *et al.*, 2011). They are therefore very important for continuous production of food and important forest plant species in the tropics.

2.3 Potential health risks of bats to humans

Close contact between bats and humans or domestic animals is a principal cause of disease emergence, and such contact has become more frequent (Wong *et al.,* 2007) due to increased hunting, habitat loss and agricultural intensification (Epstein *et al*., 2006; Leroy *et al.*, 2009). The constant improvement in transportation and the mass movement of people all over the world have also caused diseases to spread easily as compared to when movement was limited some decades ago. In Ghana, both urban and rural dwellers are at risk of disease transmission since bats are found everywhere. Due to destruction of the habitat of bats for human settlement and agricultural development, bats have begun to move into urban areas in search for food (FAO, 2011). As they forage, they come close to human settlements. This results in frequent interactions between bats and humans, serving as platforms for transmission of pathogens from bats to humans and other animals.

Fruit-eating bats in the family Pteropodidae usually do not eat a whole fruit with its palp but rather squeeze out the juice then discard the palp and seeds (Morrison, 1980). These food remnants may be left in the backyard of people where they are picked up by pets exposing these pets to various diseases that these bats may have harboured (Calisher *et al.*, 2006). These pathogens are subsequently transmitted to humans (Calisher *et al.*, 2006) (Figure 2.1). Insect eating bats may also forage into urban areas to feed on swarming insects that they find around street lights as well as lights in the porches and backyards of homes. This is another means by which there could be frequent interactions between humans and bats. Hunting of fruit bats for food also brings humans into direct contact with bats. Other animals that may have come into contact with bats and may be potential source of infection, are also hunted for food (Leroy *et al.*, 2009). Field studies showed that most bats when handled may become

aggressive and attempt to bite. Thus, bites become a means of transmission of pathogens especially for the viral pathogens when live bats are handled. Hunters are at risk of getting infected through bites of infected bats, also through the processing of raw game meat for consumption that exposes them to contamination with bodily fluids and tissues of the animal (Georges *et al.*, 1999).

Infection can also occur through contact with contaminated materials from the intestinal tract or faeces of an infected animal. Among certain tribes in Ghana, the intestinal contents of some wild animals are used to prepare meals for consumption. If handlers of such animals or meat are do not wash their hands properly prior to eating or preparing food, the food may be contaminated and transmission may occur on consumption (WHO, 2014b). Another important mode of potential disease transmission especially in Africa, is the burial practices of tribes. Most African cultures require their dead relatives to be bathed and embalmed before burial; this is a sure way of pathogen transmission. The outbreak of the Ebola virus was aggravated by burial practices among families who insist on burying their dead which they do in an improper way (Leroy *et al.*, 2009). This is most prevalent in the rural areas.

External parasites harboured by bats are also important vectors of disease-causing pathogens (eg. *Rickettsiae* sp., Pader *et al.*, 2012; *Plasmodium* sp., Schaer *et al.*, 2013). These parasites transmit pathogens between hosts. For instance, Sonenshine (1991) indicated that bat ticks transmit a diverse range of pathogens belonging to the genera *Borrelia, Rickettsia, Francisella, Ehrlichia, Anaplasma, Cowdria,* and *Coxiella,* causing conditions such as paralysis, toxicoses and allergies especially in humans. Blood feeding arthropods harboured by bats also transmit *Bartonella* sp. a gram-negative bacteria that infect the erythrocytes of

vertebrates (Chomel *et al.*, 1996; Chang *et al.*, 2001; Comer *et al*., 2001). Bat flies have been implicated in the transmission of some genera of parasites that are closely related to the *plasmodium* parasites which cause malaria in humans. By comparing parasite DNA, Schaer *et al.* (2013) were able to establish a phylogenetic tree for haemosporidians parasites in bats, showing that bats were the first mammal host to this group of parasites. This implies that the parasites later switched from bats to rodents and primates.

Another infection, Histoplasmosis, caused by the fungus *Histoplasma* sp. that lives in the droppings of bats results in various degrees of disease symptoms in humans. People get infected with histoplasmosis when they breathe in the microscopic fungal spores from the air. This causes fever, cough and fatigue, and in severe cases, splenic or pulmonary calcification, lung nodules or mediastinal abnormalities (Schwarz *et al*., 1955).

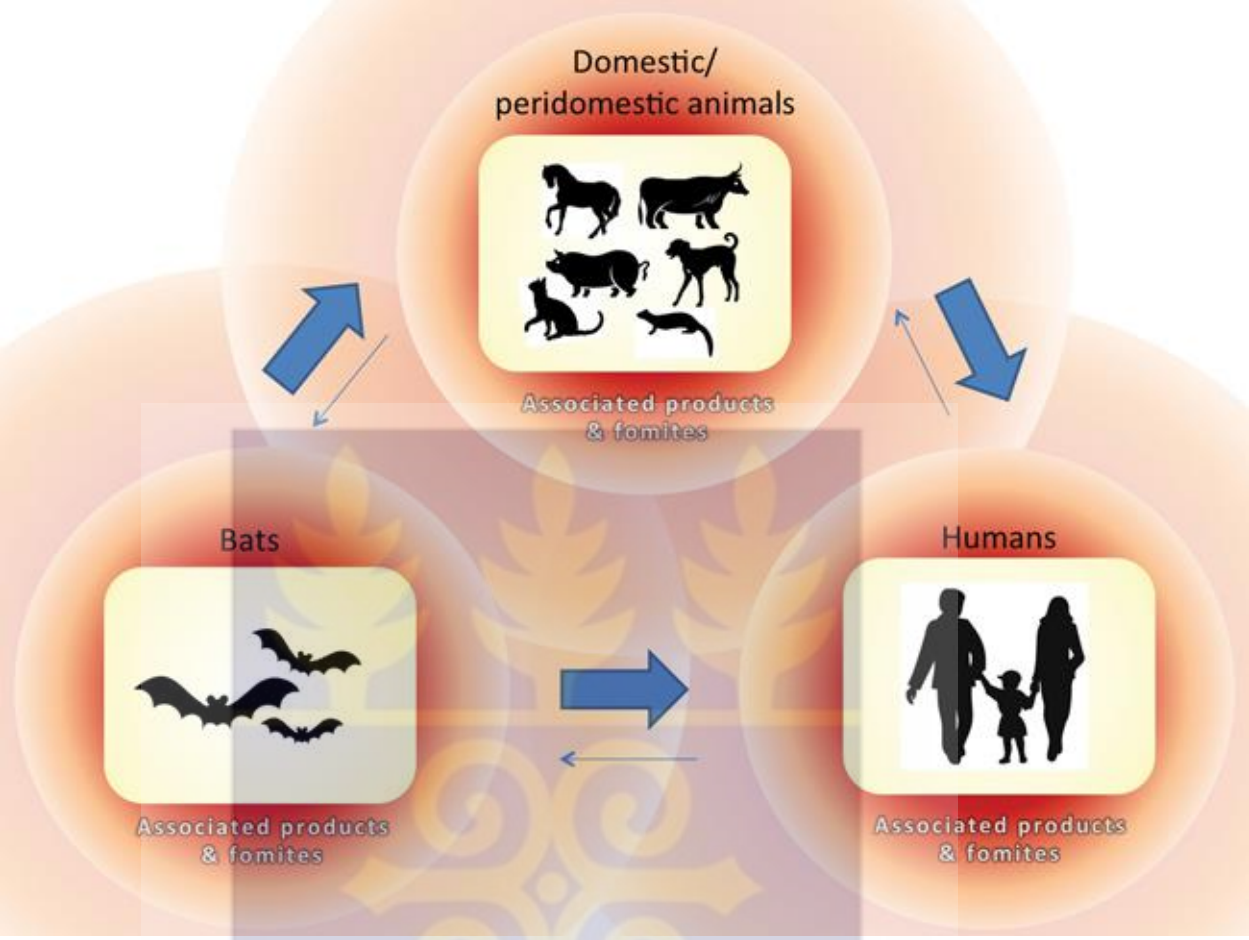


Figure 2.1: Possible routes of disease transmission between bats, domestic animals and humans. Thick arrows represent the most significant pathways for bat-associated EIDs. Thin arrows represent pathways about which less known or less common. (Kuzmin *et al.*, 2011).

2.4 Parasites

More than half of the human population live in misery and pain due to parasitic infections and suffer vast economic losses (Ambrosio and De Waal, 1990). Estimates suggest that only a six per cent reduction in disease, caused by parasites, could provide food for an additional 250 million people (Ristic and Montenegro-James, 1987). This highlights the significance of the influence of parasites on the wellbeing of human populations. They are found almost

everywhere and just like every living thing, also strive to survive even the harshest of conditions by trying to adapt to the defensive system of their hosts. Most ectoparasites and all endoparasites, by definition, are entirely dependent on their hosts for survival, inhabiting their exposed areas or internal organs, respectively (Ter Hofstede *et al*., 2004). Hence, parasite distribution and ecology is largely determined by the distribution and habits of their hosts (Wood, 2012).

Bats harbour an unusual number of ectoparasites as compared with many other mammals (Lewis, 1983) with a number of them being specific. Bat flies, for example, spend most of their life on their hosts, only leaving their hosts as larvae and so follow their hosts' geographical distribution closely (Marshall, 1982; Dick and Gettinger, 2005). The family of flies (Streblidae) is extremely specialized such that some species have lost their ability to fly during evolution and live now as 'bat lice' in the fur of bats (Wund and Myers, 2005). Mites are similarly host-dependent and complete their entire life-cycle on the body of their host (Christe *et al*., 2000). For example family Spinturnicidae are host specific and only occur on bats.

Bats also serve as host for a number of endoparasites including protozoans (*Plasmodium* and *Trypanosoma* species) (Schaer *et al.*, 2013) and platyhelminthes (cestodes, nematodes and trematodes) (Okafor *et al.*, 2004), but usually show no symptoms to the high burdens of endoparasites and pathogens that they harbour and transmit (Wenzel *et al.*, 1996).

2.4.1 Ectoparasites of bats and the diseases they transmit

Ectoparasites are organisms that inhabit the skin or outgrowths of the skin of another organism (the host) for various periods, and may be detrimental to the latter (Hopla *et al.*,

1994). Many common ectoparasites such as ticks, mites and fleas cause significant and serious infections in humans and other animals both domestic and wild.

Bats roost in a wide variety of structures from foliage and leaf tents to caves (Patterson *et al*., 2007), moving frequently between perches and interacting with many other bat species during their foraging, roosting and reproductive activities (Dick and Patterson, 2007). This offers parasites many opportunities to find new hosts and disperse among the bats in the roost. The arthropod ectoparasites of bats belong to five orders: Siphonaptera (fleas), Diptera (flies), Hemiptera (true bugs), Dermaptera (earwigs), and Acari (ticks and mites) (Whitaker, 1988) and comprise of more than 687 species (Marshall, 1982). With so many arthropod species intimately associated with bats, it is not surprising that they harbour many pathogens.

2.4.1.1 Bat flies

Bat flies are most closely related to tsetse flies (Glossinidae) and Hippoboscid flies and all belong to the same super family, Hippoboscoidea (Dittmar *et al.*, 2006). They consist of two families, Nycteribiidae most commonly found in the Eastern Hemisphere and, Streblidae (Order Diptera) predominantly in the Western Hemisphere (Dittmar *et al.*, 2006). Nycteribiid bat flies have highly specialized morphological characteristics including absence of eyes, winglessness, dorso-ventrally flattened bodies and heads that fold against the thorax when at rest (Dick and Patterson, 2006). The dorso-ventrally flattened bodies aid the fly in easy movement through the fur of its host while the folded head is rotated forward for feeding. Streblidae (Diptera: Hippoboscoidea) are obligate ectoparasites of bats (Dick and Patterson, 2008) and, consequently, are found primarily in tropical areas with relatively few species in the sub-tropical and temperate zones (Wenzel *et al*., 1966).

Bat flies are known to transmit various pathogens to animals and humans. Given the fact that bat flies occasionally bite humans (Lloyd, 2002; Wenzel *et al.*, 1966), it is theoretically possible that bat flies could transmit pathogens and diseases to humans. In a study carried out by Azner-Lopez *et al.* (2013), all samples of nycteribiids tested positive for rhabdovirus viral RNA. This indicates the possibility of transfer of this virus. The bacteria *Bartonella* sp. has been detected in a bat fly *Cyclopodia greefi greefi* collected in West Africa, specifically Ghana and two islands in the Gulf of Guinea (Billeter *et al*., 2012). Kamani *et al*. (2014) also detected *Bartonella* sp. in nycteribiid flies sampled in Nigeria. Other studies have confirmed the presence of *Bartonella* spp. in bat ectoparasites from Egypt and the United States (Loftis *et al*., 2005; Reeves *et al*., 2005; 2007). Streblid bat flies were not known to transmit pathogens but they feed repeatedly on multiple bats and often fly from host to host in roosts and as a result, could serve as mechanical or biological vectors of pathogens, while maintaining pathogens within bat colonies. Bat flies have however recently been implicated for transmission of malaria-like pathogens (Poinar, 2011).

2.4.1.2 Bat mites

Mites are the most ubiquitous, bothersome pests. A few species including scabies mites, trombiculid larval mites and animal and plant mites are of medical importance (Diaz, 2010). Mites have exploited a wide array of habitats, but because of their small size, they are hardly noticed. Many live freely in the soil or water, but there are also a large number of species that live as parasites on plants, animals (bats not exempted) and some even feed on mold (Halliday *et al.*, 2000). Various mite species could be found in the ears, around the eyes or on the wings (Walter, 1996) of bats feeding on the skin or hair debris of their hosts. Some

live within the skin or mucous membranes of body cavities such as the nostrils and mouth where they attach themselves with highly modified legs. Mites are closely related to ticks, but they are tissue-juice feeders, not blood-feeders, and do not transmit as broad a variety of infectious microbial diseases (Service, 1996).

Bats can be infested with various mite species including mites of the family Spinturnicidae, which are most abundant, and many species of Macronyssidae which are all exclusive parasites of the bats during all life stages (Krantz and Walter, 2009).

Mites serve as biological or mechanical vectors for disease-causing pathogens. They can cause several allergic responses or diseases including fever, asthma and eczema. According to Diaz (2010), only the Asian and Eurasian *Leptotrombidium* species of trombiculid larval mites (or chiggers) can transmit scrub typhus in endemic regions of Asia, Eurasia, and the South Pacific. On the other hand, only the house-mouse mite can transmit *rickettsial* pox in both urban and rural settings worldwide. However, this may not be conclusive as zoonotic diseases continue to emerge and widen their scope of spread of diseases across species boundaries.

2.4.1.3 Bat ticks

After mosquitoes, ticks are the second most important arthropod vectors of diseases to both humans and other vertebrates (Edlow and Kulkarni, 2008; Dalgic *et al*., 2010). Ticks remain responsible for severe economic losses both through the direct effects of blood sucking and indirectly as vectors of pathogens and secretion of toxins (Jongejan and Uilenberg, 1994). They are a major concern to human health and productivity of livestock. Changing environmental conditions, including climate change, land-use patterns, wildlife populations

and agricultural practices, are all acting to alter host and tick ecology and their geographical distributions, leading to new regions of tick activity, overlapping distributions and emergence of diseases (Van Overbeek *et al.*, 2008; Randolph, 2010). World-wide losses due to diseases transmitted by ticks and the costs of tick control have been estimated to be in the range of several billion US dollars ($109 billion) annually (FAO, 1984; McCosker, 1979). However, the major losses caused by ticks are due to their ability to transmit protozoan, rickettsial and viral diseases of livestock, which are of great economic importance world-wide (Jongejan and Uilenberg, 1994). In Africa, tick- borne diseases and tick infestations are among the most commonly documented causes of morbidity in humans and livestock (Phiri *et al*., 2010).

Ticks (Ixodid/Hard and Argasid/Soft) have three active parasitic stages as larva, nymph, and adult (Sonenshine, 1991) and their life cycle takes up to one year to complete. They rely on blood meals for food and development. Argasid ticks may go through several nymphal stages, requiring a meal of blood each time (Aeschlimann and Freyvogel, 1995).

Ticks cause anaemia and toxicosis (e.g. tick paralysis due to tick saliva) in the host (Sonenshine, 1991). The bites of soft ticks, notably *Argas* and *Ornithodoros* species, which are common ticks of bats, cause irritation, blisters, bruising as well as severe pruritus in humans (Estrada-peña and Jongejan, 1999). *Argas* sp., found commonly on bats, transmits a greater variety of pathogenic micro-organisms than any other arthropod vector group (Kohls and Hoogstraal, 1961; Schwan *et al.*, 1992). Argasid ticks transmit mainly viral pathogens with a few bacterial pathogens which cause severe diseases in humans and animals. The argasid-borne bacteria are almost exclusively *Borreliae*, which cause relapsing fever in

humans (Manzano-Román *et al.*, 2012). Another bat tick, *Carios kelleyi* was found to harbour the bacteria *Bartonella henselae* and *Rickettsia* sp. (Loftis *et al*., 2005). Apart from pathogenic bacteria transmitted by ticks, they are also capable of transmitting pathogenic piroplasms (e.g. Babesia and Theileria species) (Bishop *et al.*, 2004; Florin-Christensen and Schnittger, 2009).

The ability of ticks to transmit a great variety of infectious diseases to humans is a major public health concern worldwide (Awwad *et al.*, 2002). *Argas boueti*, a species usually associated with bats, was found on humans in Egypt (Hoogstraal, 1956). *Argas vespertilionis*, a parasite of bats, can be highly aggressive in man and has been isolated from humans in Africa (Hoogstraal, 1956), the former Soviet Union (Galuzo, 1957) and Iraq (Keirans, 1984). In 1966, *Coxiella burnetii*, the agent of Q fever, was detected in *Argas vespertilionis* ticks collected from southern Kazakhstan (L'vov, 1973). An arbovirus named Issyk-Kul virus was isolated from bats and *A. vespertilionis* ticks in Kyrgyzstan (L'vov, 1980). A few years thereafter, Issyk-Kul virus was isolated from a scientist who had become infected while conducting field work in the Kumsangir district of southern Tajikistan (Gardner and Molyneux, 1987). This heightens the likelihood of pathogen transmission from bat ticks to humans. It is likely that ticks are host to a larger diversity of microbes which are yet undiscovered.

2.4.1.4 Bat fleas

Bat fleas are small, laterally flattened, wingless and highly specialized insects that live in the fur of its host. They feed exclusively on blood and are of great importance as vectors of pathogens in many parts of the world (Bitam *et al.*, 2010). About 2574 species belonging to

16 families and 238 genera have been described, but only a minority is synanthropic, that is live in close association with humans (Lewis, 1999).

Fleas are mainly vessel feeders, thus damaging blood vessels directly (Bitam *et al*., 2010). This therefore makes them one of the best candidates for direct pathogen dispersal. The two commonly known ways of pathogen transmission by fleas are by oral route through regurgitation of blood meals, or by faecal route, by contaminated faecal pellets (Bitam *et al*., 2010). Many fleas are associated with domesticated animals rather than humans, and may thus have economic implications, rather than direct effect on human health. An abundance of human-associated fleas (*Pulex irritans, Ctenocephalides felis* and *Xenopsylla cheopis*) have been described in human dwellings in plague-endemic regions in Africa (Laudisoit *et al.,* 2007; Eisen *et al.,* 2008).

The most severe infection spread by fleas is plague, caused by *Yersinia pestis* (Stenseth *et al.,* 2008). Plague is a zoonotic disease primarily affecting rodents but can also affect humans (Bitam *et al.,* 2010). Small outbreaks continue to occur throughout the world with around 2000 cases reported annually (Gage and Kosoy, 2005). Plague has recently be recognized as a re-emerging disease and remains a serious problem for international public health, especially in Africa (Bertherat *et al.,* 2007; Stenseth *et al*., 2008; Neerinckx *et al*., 2008). A case in point is the outbreak of the disease in Madagascar (Friedman, 2014). *Yersinia pestis* has been the cause of three pandemics (Pollitzer, 1954; Raoult *et al*., 2000; Drancourt *et al*., 2004). Fleas are also known as vectors of murine typhus (endemic typhus, *Rickettsia typhi*), and play a role in the transmission of rural endemic typhus (*Rickettsia prowazekii*) in the USA (WHO, 1989). Fleas are known to harbour and sometimes transmit *Bartonella* sp., including *Bartonella henslae*, the agent of cat-scratch disease (Chomel *et al.,* 2006; Billeter

et al., 2008). Additionally, fleas are hosts of helminths: *Dipylidium caninum* and *Hymenolepis diminuta* parasites of carnivores and rats respectively (Duchemin *et al.*, 2006). Finally, in tropical areas, tungiasis caused by *Tunga penetrans* is a human disease directly linked to the parasitism of humans by fleas (Reiss, 1966). Tungiasis leads to irritation, blood loss and discomforts (Reiss, 1966). Flea infestation can also lead to hair loss as a result of frequent scratching and biting by the animal, and can cause anaemia in extreme cases (Mullen *et al.*, 2009).

2.4.1.5 Bat bug

Bat bugs are blood-sucking insect parasites that feed primarily on the blood of bats. Bat bugs include members of the families Cimidae (eg. *Cimex lectularius* and *Afrocimex constrictus)* and Polyctenidae. The family Cimicidae (Heteroptera) is a widespread family containing about 90 species belonging to 23 genera in six subfamilies (Péricart, 1996). About two thirds of the species of the family Cimicidae are associated with bats, and bats have been suggested to be the original host group of the family (Horváth, 1913).

Bat bugs are closely related to bed bugs and are similar in appearance so they are often mistaken for bed bugs. Bat bugs are most abundant in the roosts of colonial bats. Adult cimicids will take blood meals lasting 10 to15 minutes and then leave the host to digest the meal (Wilson and Galloway, 2002). Cimicids can survive up to a year without taking a blood meal which allows them to stay in their host's roosts after the bats have left to hibernate (Bartonicka and Gailsler, 2007). Blood feeding however is required by the female in order to produce eggs.

Bat bugs are vectors of *Trypanosoma* sp. and *Bartonella* sp. (Loftis *et al.*, 2005; Reeves *et al.*, 2007). Under experimental conditions, *Cimex* sp. has been shown to transmit *Trypanosoma cruzi*, the causative agent of Chagas' disease (Hoare, 1972). Apart from bat bugs transmitting pathogens, the saliva from the bug causes severe itching and allergic reactions in humans.

2.4.2 Endoparasites of bats

The endoparasites reported in bats include protozoans (*Plasmodium* sp., *Nycteria* sp., *Hepatocystis* sp. and *Polychromophilus* sp.) (Schaer *et al*., 2013), trypanosomes (Thomas *et al.*, 2007) and helminths (trematode s, cestodes and nematodes) (Okafor *et al.*, 2004; Nogueira *et al.*, 2004). Endoparasites are very evasive parasites which find various ways of evading the immune system of their hosts. They lodge in organs such as the heart, liver and lungs of their host and also in fluids such as the blood and lymph and in fact every part of its host. The well adapted nature of endoparasites can be seen in the adult *Toxocara pteropodis* which are found only in the intestines of suckling young flying fox which pass the eggs in faeces (Nelson, 1989). The adult bats get infected by ingesting faeces from foliage in the roosting habitats. The eggs of these helminths hatch and move to the liver of adult male bats and mammary glands of the adult female bats.

2.4.2.1 Malaria parasites

Bats are a very important reservoir for malaria causing pathogens. Whereas there are number of numerous studies on human malaria, studies on bat haemosporidian parasites have been largely neglected since the eighties (Megali *et al.*, 2011). Haemosporidia infecting bats fall

into four genera, *Plasmodium*, *Hepatocystis*, *Nycteria* and *Polychromophilus* (Garnham and Heisch, 1953). The three first genera occur only in the Old World tropics of Asia and Africa, whereas *Polychromophilus* also occurs in temperate zones in Europe and to a lesser extent in the New World (Adam and Landau, 1973; Garnham, 1973). Malaria is caused by a handful of species of parasites in the genus *Plasmodium* through the bite of mosquitos and remains a widespread vector-borne infectious disease, affecting almost half a billion people every year around the planet. Over the years malaria infection has been of great importance especially in the tropics. According to the latest estimates, 198 million cases of malaria occurred globally in 2013 and the disease led to 584, 000 deaths (WHO, 2014c). Majority of those infected were children under the age of 15 years (WHO, 2014c) and pregnant women. Haemosproridia use at least seven families of blood sucking dipteran insects, as vectors (Levine, 1988). Bat flies of the family Nycteribiidae transmit bat malaria globally, however their closely related members, streblids, have never been implicated as vectors of bat malaria (Garnham, 1966; Rosin *et al*., 1978). Scientists at the Max Planck Institute for Infection Biology, the Museum für Naturkunde in Berlin and the American Museum of Natural History have identified four genera of parasites that are closely related to the malaria pathogen in West African bats (Schaer *et al.*, 2013). One of them is the genus *Plasmodium*, which also includes the species that cause malaria in humans. This *Plasmodium* species in bats are very similar to that found in rodents. The researchers examined 31 bat species from the West African forest in Guinea, Liberia and Ivory Coast with regard to parasites that infect red blood cells (Schaer *et al.*, 2013). Forty per cent of the approximately 270 examined bats carried parasites of the genus *Plasmodium*, Polychromophilus, Nycteria and Hepatocystis. According to the study, at least two species of *Plasmodium* can be found in bats. The high

diversity of parasites as well as the high proportion of individuals that are infected with the parasites suggest that this is yet another example of the unusually high tolerance of these flying mammals to pathogens (American Museum of Natural History, 2013).

2.4.2.2 Trypanosomes

The African trypanosomes comprise a group of important and complex pathogens, affecting animal and human health in much of sub-Saharan Africa (Cox *et al.*, 2005). According to Dias (1936), trypanosomes of bats have been known since 1898 when they were first isolated from three species of vespertilionid bats (*Miniopterus schreibersii, Vespertilio murinus*, *Vesperugo noctula*) in Italy and described. Trypanosomes (genus Trypanosoma) are widespread blood parasites of vertebrates, usually transmitted by arthropods or leech vectors (Hamilton *et al*., 2004). Trypanosomes have been recorded in more than 70 species of bats (Chiroptera) from a wide range of families (Lima *et al*., 2012). Although little is known about the transmission of trypanosomes in bats, the arthropod bug of the family Cimicidae, have been implicated as vectors. Despite the potential epidemiological significance of bats as reservoir hosts for these trypanosomes, information about their vectors, and specifically how infections might be obtained and spread, is mostly lacking (Thomas *et al*., 2007).

Most species of bat trypanosomes are found within the subgenera Megatrypanum and Schizotrypanum which are the only sub-genera known to infect bats (Cavazzana *et al*., 2010). Species of Trypanosomes (Schizotrypanum) reported in bats include: *Trypanosoma vespertilionis* in Europe, the Americas, Africa and Asia; *T. dionisii* and *T. pipistrelli* in the Old World, particularly European countries; *T. pteropi* and *T. hipposideri* in Australia; *T. hedricki* and *T. myotis* in North America; *T. phyllostomae* and *T. cruzi marinkellei* in Central

and South America; and *T. cruzi* in all Latin America, as well as South and Southwest of the USA (Hoare, 1972; Marinkelle, 1976; Molyneux, 1991). *Trypanosoma cruzi*, and a number of other trypanosomes cause Chagas disease in humans.

2.4.2.3 Helminths

Although generally not considered to be pathogenic, gastrointestinal helminths are known to influence host immune status (Maizels and Yazdanbakhsh, 2003) and hence may have a role in the overall health status of an individual animal and may also influence co-infection with other parasites. Several of helminths reported in wild non-human primates have the potential to be transmitted to humans (Muller-Graf *et al*., 1996; Munene *et al*., 1998; Krief *et al*., 2010). The prevalence and incidence of helminths seem to be strongly affected by the feeding habits and foraging strategies of bats (Coggins, 1988) and so the greater a bat's specialization on a particular prey item the more likely they are to become parasitized by the specific helminth using that prey item as an intermediate host (Hilton and Best, 2000). Insect-eating bats therefore usually have higher helminth diversity and abundance than fruit-eating bats. Up until now, most studies that have been done on helminth fauna with respect to bats are on insectivorous bats (Okafor *et al.*, 2004; Nogueira *et al*., 2004).

The bat gastrointestinal tracts seem to be dominated by trematodes other than cestodes and nematodes (Ricci, 1995; Shimalov *et al.*, 2002). Trematodes are predominantly found in those insectivorous bats that are prone to ingest infected aquatic insects (García-Vargas *et al*., 1996; Pérez-Ponce de León, 2001) than fruit-eating bats. Okafor *et al*. (2004) however found nematodes to be abundant in the insect-eating bat *Tadarida nigeriae*. The few helminths of fruit-eating bats include *Nycteridocoptes rousettus* (Saoud and Ramadan,

1976), *Hasstilesia tricolor*, *Vampirolepis elongates*, *Capillaria* sp. and *Cheiropteronema globocephala* (Nogueira *et al*., 2004).

2.5 Host-parasite associations

Bats can be co-infested with different species of ectoparasites at a time. These could include ticks, mites and bat flies. Several bat fly species for instance can coexist on a single host, typically partitioning the bat's fur and wing membranes (Patterson *et al*., 2008). This however is not the same in the case of Spinturnicid mites (Bruyndonckx *et al.,* 2009) with usually a single mite species on a host.

Parasitism in bats is influenced by several factors including the sex of the bat, age of the bat, roosting behavior, physiology of the bat and seasonal changes (Lučan, 2006). Many ectoparasites of bats are present on their host all year-round while others infest their hosts only during critical stages of the host's life cycle such as gestation or lactation (Zenon and Hugh, 2011). The latter is seen in the reproductive cycle of many species of mites in which presence is stimulated by host pregnancy hormones (Dick *et al*., 2003; Lourenço and Palmeirim, 2007). Hence lactating female bats in maternity colonies host a higher intensity and prevalence of ectoparasites than non-lactating females and male bats (Christe *et al*., 2000; Reckardt and Kerth, 2009). Since the young of the host are always attached to the female, there is a vertical transmission of ectoparasites to the young when they are born. The young bats may be less efficient in grooming to decrease ectoparasites load and may have a naïve immunoresponse hence hosting high ectoparasite loads (Gannon and Willig, 1995; Christe *et al.,* 2000). On the contrary, other studies found that male bats carried heavier parasite load than female bats (Moore and Wilson, 2002; Morand *et al*., 2004).

Ectoparasite incidence and prevalence is also influenced by temperature in both the temperate and tropical regions. Low temperature and humidity may lead to mortality of ectoparasitic insects (Marshall, 1982). In both of these regions, the pattern of many infectious diseases are driven by seasonal changes with more ectoparasite infestation in warmer seasons than cooler seasons (Zhang *et al*., 2010). Roosting behaviour also influences parasite loads (Uhrin *et al*., 2010) in that, large group sizes usually have a higher parasitic load than smaller groups or solitary bats. Consequently, cave dwelling bats and highly gregarious ones would be expected to have higher densities of parasites than less gregarious bats. For instance, cave dwelling *Rousettus aegyptiacus* (Rosevear, 1965) would be expected to have high burdens of ectoparasites than solitary or less gregarious bats such as *Epomops buettikoferi*. *Epomophorus gambianus* a highly gregarious species is also expected to have higher ectoparasite infestation compared to solitary bats. Apart from the gregarious nature of some species of bats, the warm temperature in roosting habitats favours reproduction of the ectoparasites. The physiological status of the host also may determine the type and level of infestation of ectoparasites of bats (Mooring *et al.*, 2006).

The effect of host factors such as sex or age, on the composition and structure of helminth assemblages in small mammals is relatively unknown and with respect to digenean trematode infection, there are no reports implicating any strong influences due to host age, or reproductive status (Feliu *et al.*, 2006).

2.6 Effects of parasites on bats

Bats often host heavy loads of ectoparasitic arthropods than most mammals (Zahn and Rupp, 2004). The effect of parasitism on the condition of the host has been the focus of many

studies (Lucân, 2006; Patterson *et al.*, 2008; Sarasa *et al.*, 2011). While under some circumstances, the authors found a significant effect (Brown and Brown, 1986; Neuhaus, 2003; Brown and Brown, 2004; Whiteman and Parker, 2004), others concluded that the body condition of the hosts was independent of parasitic load (Tompkins *et al*., 1996; Perez-Orella and Schulte- Hostedde, 2005). Parasitic infections may however reduce competitive fitness (Brassard *et al*., 1982; Scott, 1988), influence population cycles (Hudson and Greenman, 1998) and regulate host population abundance (Anderson and May, 1978). Regulation of host abundance by ectoparasites has been observed in many species; for example voles (Okulova and Aristova, 1973), horses (Rubenstein and Hohmann, 1989), barn swallows (Møller and Rózsa, 2005) and bats (Lourenço and Palmeirim, 2007). With regards to host fitness, a strong negative correlation was found between the number of *Spinturnix psi* on the bent-wing bat (*Miniopterus schreibersii*) and the host's body condition (Lourenço and Palmeirim, 2007). Poor condition of hosts may in turn increase the susceptibility of hosts to predation, diseases and starvation, and consequently reduce survival or reproductive success of the host (Brown and Brown, 2004).

Some ectoparasites may appear not to affect their hosts physically, but may have detrimental effects on other life history traits. The host may be affected positively or negatively depending on the type of ectoparasite it is affected with. For instance bat fly bites are painful to humans causing sore or lesions, but bats exhibit no reaction to the bite of bat flies (Wenzel *et al.*, 1966). This however cannot be used as a measure to conclude that bats are not affected by bat fly parasitism. Although bats may not be affected physically, they may be affected in their grooming behaviour, diet preference and their choice in habitat preference. On the other

hand, adult *Myotis myotis* artificially infested with *Spinturnix myotis* drastically decreased their sleep in favour of grooming (Giorgi *et al*., 2001).

Avoidance and removal of ectoparasites through grooming, are two behavioural strategies animals use to reduce the cost of parasitism. Bats may alter their habitat in response to ectoparasite infestation in a bid to avoid high burdens of parasites and consequences of parasitism (Patterson *et al.*, 2007). Hosts also avoid ectoparasites by living in habitats that are unsuitable for the parasite. Evidence of habitat selection as a defence mechanism against ectoparasites has been reported for a variety of animals (Hart, 1992). These habitats however may be exposed to more predators and other diseases, thereby reducing the survival rates of hosts. Self-grooming in bats is important in controlling ectoparasite load and may be the main cause of ectoparasite mortality (Marshall, 1982; Wilkinson, 1986). Grooming also helps in maintaining the condition of the fur of feathers and strengthens social bonds.

On the contrary, although grooming aids in removal of ectoparasites, it may affect negatively the benefits of bats in the ecosystem in terms of pollination and insect control. Lesser important plant species will be pollinated and insect (pest) populations will increase. Ectoparasitism abundance may also cause bats to groom more frequently thus decreasing the time and energy for reproduction and feeding.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study areas

The study sites were the '37' Military Hospital, an urban site, in Accra, Buoyem and Tanoboase Sacred Grove in the Brong Ahafo Region and Ve-Golokuati in the Volta Region which were rural sites (Figure 3.1). All areas had large bat roosts with high potential for human-bat interactions.

3.1.1 '37' Military Hospital

The '37' Military Hospital is located in the Greater Accra region of Ghana on the coordinates N 05° 35.134′, W 000° 11.069′ and is the second largest hospital in Ghana. The '37' Military Hospital is a busy area with settlements, schools and lots of human activities. Activities in the area include trading, public transport operations and food vending. A large colony of bats roosted in the trees surrounding the hospital. According to local legend, the bats followed an ailing chief of Kyebi, a town in the Eastern Region of Ghana, who was admitted at the hospital and never returned after the demise of the chief. It is said that the bats still await the chief to accompany him back home. The main trees at the '37' Military Hospital were the Neem tree (*Azadirachta indica*) in which the bats roost.

Accra has a tropical climate characterized by a rainy season, known as the wet season, and the harmattan season also known as the dry season. The average annual rainfall is about 730 mm, which falls primarily during the two rainy seasons. The major rainy season begins in April and ends in mid-July, whilst the minor rainy season occurs in October. The dry season usually occurs between the months of December and January. There is very little variation

in temperature throughout the year with mean monthly temperatures ranging from 24.7 °C (76.5 °F) in August which is the coolest month to 28 °C (82.4 °F) in March which is the hottest month, with an annual average of 26.8 °C (80.2 °F) (AMA, 2006).Most animals have been pushed inland because of the rapid expansion of settlements in the Metropolitan area (AMA, 2006).

3.1.2 Buoyem

Buoyem is a village located in the Brong-Ahafo region of Ghana and located approximately 8km from Techiman (Briggs, 2010). It is located in the North constituency of the Techiman district of Ghana on the coordinates N 07º 43.416′, W001º 59.266′. The population of Buoyem is approximately 3,500 (Ghana Population Census, 2000) and most of the people in the population speak Brong as their mother tongue.

Buoyem has two main vegetation types, the moist semi-deciduous forest and the guinea savannah woodland. This region is a transition zone between the dry savannah woodland of the northern region and the forested zone of the southern region of Ghana. The climate here consists primarily of two seasons, the dry season and the rainy season. Rainfall is usually heavy in the months of June and July as well as in the months of September and October. During the dry season which is usually from November to February, the climate in these areas is predominantly hot with dry conditions due to the Harmattan wind which blows during this season. Temperatures in this region range between 18.3ºC and 29.4ºC while annual rainfall is between 1000mm to 1400mm.

The Buoyem town is home to the Buoyem Sacred Grove through which the River Mprisi also flows. The historic Bono Shrine is also located in this village. The groves contain a

diverse fauna including a large colony of fruit bats where over 20,000 roost in underground caves (SDSS, 2000). The forests and bushes here support baboons and antelopes (Yeboah, 2014). Rodent and avifauna are high in numbers and, typical of such transitional vegetation, offers varied habitats for both forest and grassland species.

3.1.3 Tanoboase Sacred Grove

Tanoboase is also a village in the Brong Ahafo Region located about 15km north of Techiman, along the Techiman-Kintampo road. The Tanoboase sacred grove, located on the coordinates N 07º 39.942′, W 001º 51.448′, is surrounded by naturally formed rocks that are high and almost appear artificially arranged (Plate 3.1). Majority of the people here are subsistence farmers who grow cash crops such as cashew nut, cocoa and mango. Food crops grown include maize, groundnut, tomatoes, yams, tiger nuts, garden eggs and several other foodstuff, with cassava being the main crop.

The vegetation cover at the Tanoboase Sacred Grove also comprise two main vegetation types, the moist semi-deciduous forest and the guinea savannah woodland. The climate and temperatures here is similar to that of Buoyem. The grove contains a variety of mammals such as Pata monkey (*Erythrocebus patas*), baboons, monkeys and antelopes. The grove also has a variety of plants, trees, birds and butterfly species.

Plate 3.1: Naturally formed rocks at the Tanoboase Sacred Grove

3.1.4 Ve-Golokuati

Ve-Golokuati is a town situated in the Volta Region of Ghana (coordinates N 06º 59.851′, E 000º 26.218′) off the main Ho-Hohoe road. It is about 58 km from Ho, the capital of the Volta Region. Ve-Golokuati is a typical 'Ewe' land where the inhabitants are known as the Ewe people and speak the Ewe language. The people celebrate the Lukusi festival dubbed Dodoleglimeza by the people. The main occupation of this village is farming where the farmers cultivate food crops such as cassava, yam, okro, garden eggs and rice. Some of the inhabitants are also traders.

The annual rainfall total ranges between 1100mm and 1500mm, averaging 1300mm. Ve-Golokuati also falls within the forest-savannah transitional ecological zone of Ghana with a rich conglomerate of fauna and flora. Fauna that can be found here include grasscutter (*Thryonomys swinderianus*), bats and various rodents.

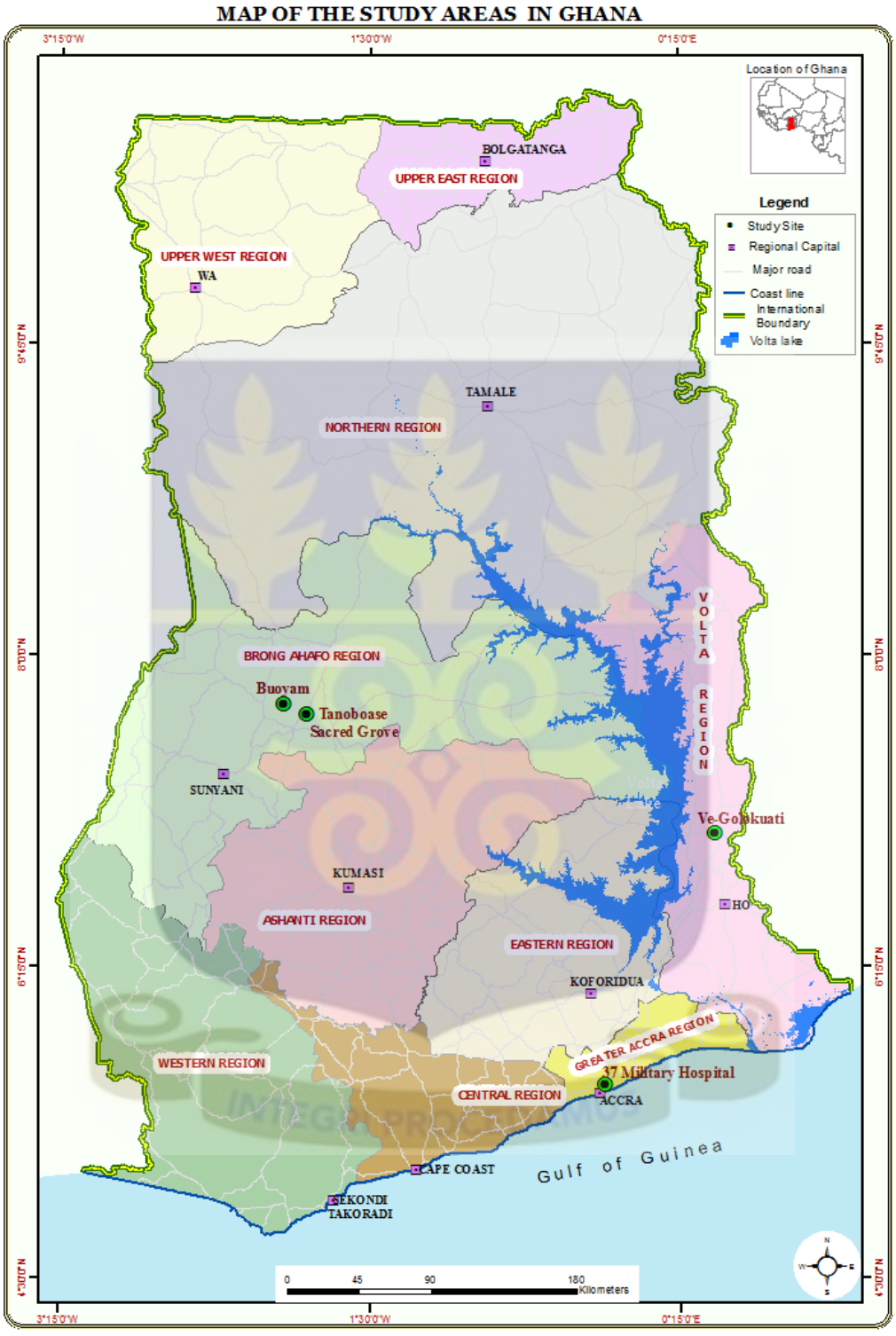


Figure 3.1: Map showing the study sites.

3.2 Data collection

3.2.1 Trapping of bats

Bats were captured in 12m by 15m mist nets with four shelves as part of an ongoing study of bat ecology under the Dynamic Drivers of Disease in Africa Conservation (DDDAC). The nets were set from 7 pm to 5 am and inspected every two hours to ensure that bats captured did not stay too long in the net struggling. Bats hunt at night for food and therefore it was necessary to work on them quickly and release them to go and feed. Captured bats, as shown in Plate 3.2, were removed and placed in aerated sacs and brought to the working area for examination for parasites. Thick garden gloves were worn while removing the bats from the nets to prevent scratches and bites.

The weight, forearm lengths, sex of the bats and reproductive status were recorded. Bats were identified with the help of a field guide 'Bats of West Africa' by Rosevear (1965) and then inspected for parasites.

Plate 3.2: A bat entangled in a mist net.

3.2.2 Sampling of ectoparasites

Individual bats were carefully handled and examined for ectoparasites (Plate 3.3). The fur, wing membranes and ears of each bat were carefully searched with a light-emitting diode (LED) lamp and visible ectoparasites were carefully picked with the fine forceps and placed in plastic eppendorf tubes half filled with 70 percent ethanol. Placing the ectoparasites in 70 percent ethanol ensured that the specimens were not dehydrated and that important features remained intact. Each sample was then labelled with a unique number and locality. This information as well as other data including the species of bat were recorded on a data sheet for future reference.

Apart from the visible ectoparasites that were picked, bats are known also to harbour ectoparasites that are so small that they are not visible to the human eye. Such parasites were collected by cleaning the whole body surface of the bat with cotton wool soaked in 70 percent ethanol (Plate 3.4). This will immobilize and pick up any microscopic parasites on the surface of the bats. The used cotton was then placed in a Ziploc bag, sealed and labelled.

Plate 3.3: A bat fly securely lodged in the fur of a bat

Plate 3.4: A bat being cleaned with 70 percent ethanol for microscopic ectoparasites

3.2.3 Sampling of endoparasites

3.2.3.1 Sampling of blood

Blood samples were collected from the propatagial vein in the wing of individual bats using a small needle to prick the vein and a small plastic disposable Pasteur pipette or capillary tube to pick a drop of blood onto a clean labelled frosted slide. A second slide was then used to make a blood smear and the blood allowed to air dry. The blood smear on the slide was then fixed immediately after drying by dipping the slide into 100 percent methanol for about two to five minutes. The slides were then allowed to air dry then kept in the slide boxes and stored in a cool and dry place for later staining.

A drop of blood from each bat was also taken and dropped on a dry filter paper as described by Omanwar *et al*. (1999) for later molecular work. The blood stained filter paper was then allowed to air dry and samples placed in a ziplock bag and kept dry with some silica gel in a cool dry place. Nose masks were worn for protection from inhalation of droplets of blood and saliva from the bats.

3.2.3.2 Sampling of faecal pellets

Faecal pellets of the bats were taken for molecular analysis for helminths. During the sampling process, faecal pellets passed out by the bats were collected and stored in eppendorf tubes half filled with 2.5% potassium dichromate (Kuk *et al.*, 2012). This is to preserve and maintain the integrity of any eggs or cysts that the faecal pellets may contain. Faecal pellets that fell on the ground or on gloves were not collected in order to prevent cross contamination of samples.

3.3 Processing of samples

3.3.1 Macroscopic ectoparasites

3.3.1.1 Morphological identification of parasites

Ectoparasites that were preserved in ethanol were examined under a dissecting miscroscope and identified with various identification keys (Kolenati, 1856, 1857; Radford, 1947; Hoogstraal, 1956; Rudnick, 1960; Yunker and Eleanor, 1961; Maa, 1962; Delfinado and Baker, 1963; Radovsky and Yunker, 1963; Radovsky, 1967; Machado-Allison and Antequera, 1971; Krantz, 1978) for mites, ticks and bat flies.

3.3.2 Microscopic ectoparasites

The ethanol soaked cotton which was used to clean the bats was washed with distilled water and 70 percent ethanol in petri dishes and observed under a light microscope for identification of parasites. Very small parasites were picked with forceps and placed unto a slide and a higher power of magnification (x100) used to observe and identify them.

3.3.3 Identification of blood parasites

3.3.3.1 Processing of blood smears on slides

The fixed slides were stained for 30 minutes using 5% prepared giemsa stain and observed under a digital microscope for parasites (protozoans and trypanosomes). Staining the slides makes the parasites easily visible for viewing. Slides were viewed using the oil immersion lens and pictures of positive samples taken for recording purposes.

3.3.3.2 Molecular identification of blood parasites (DNA exctraction procedure)

DNA of *Plasmodium* sp. was extracted from the blood spots dried on filter paper using the Chelex protocol of Wooden *et al*. (1993). About 3mm square piece of the blood blot was individually cut out using a pair of scissors. The scissors was sterilized with 70 percent ethanol in between cuts. Each 3mm sample was transferred into a 1.5ml microcentrifuge tube. One millilitre of freshly prepared phosphate buffered saline (1xPBS) of pH 7.4 was added, and the tube inverted three times and incubated at room temperature overnight. The tube was then centrifuged at 14000rpm for two minutes and the supernatant which was reddish in colour discarded. Another 1ml of 1xPBS was then added to the tube containing the filter paper, inverted several times and centrifuged at 14000rpm for two minutes. The supernatant was then discarded by pipetting with a fresh pipette tip for each sample. One hundred and fifty microliter of Polymerase Chain Reaction (PCR) water and 50µl of 20% Chelex were then added to the tube and vortexed. The DNA was extracted by incubating the tube and its content at 95 ^{o}C for 10 minutes, vortexing every two minutes. After incubation, the tube was then centrifuged at 14,000rpm for one minute and the supernatant transferred into a fresh 0.5ml microcentrifuge tube ensuring that the Chelex was not carried over. The DNA in the tube was stored at -20^{o}C until ready for use.

3.3.3.3 Polymerase Chain Reaction (PCR) (Blood Samples)

Table 3.1: Reagents in PCR mix for malaria parasites

A polymerase chain reaction was performed by adding the following in the respective volumes. Details of forward and reverse primers are as shown in table 3.3.

Reagents in PCR mix	**Volume/ µl**
Maxima Hot Start PCR Master Mix (2x)	12.5
Forward Primer (DW2)	1
Reverse Primer (DW4)	1
Template DNA	2
Nucleus-free Water	8.5
Total	25

PCR was performed using the following cycling program: 4 minutes at 95^0C; then 38 cycles of 30 seconds at 95^0C, 90 seconds at 51^0C, 2 minutes at 64^0C; and final extension for 15 minutes at 64^0C.

3.3.3.4 Agarose Gel Electrophoresis (Blood Samples)

Two percent of agarose gel was prepared by putting 2g of agarose into 100 ml of 1x TAE buffer into a conical flask. The mixture was heated until almost boiling and the agarose well dissolved. 2µl of ethidium bromide (EtBr) was added to the solution. Ethidium bromide binds to the DNA and allows one to visualize the DNA bands under ultraviolet (UV) light. The agarose gel mixture was then allowed to cool for a bit and then poured into a gel tray with the comb in place. This was allowed to sit at room temperature for about 20 to 30

minutes until it was completely solidified. After the gel had solidified, the well comb was taken out carefully so the wells were well set. Once solidified, the agarose gel was placed into the gel box (electrophoresis unit). The gel box was then filled with 1x TAE until the gel was completely covered.

A molecular weight ladder was carefully loaded into the first well in the gel. 5µl of loading buffer was added to 10µl of each sample and carefully loaded into different wells in the gel. The gel was then run at 150 volts until the dye line was approximately 75 to 80 percent of the way down the gel. DNA is negatively charged and therefore travelled towards the positive end of the gel electrophoresis apparatus. The power was then turned off, the electrodes disconnected from the power source and the gel carefully removed from the gel box. An Ultra Violet viewing machine was used to visualize the DNA fragments and photographs of the gel taken using a Gel Doc system.

3.3.4 Faecal parasites

3.3.4.1 Molecular identification of parasites

Molecular analysis as described by Guardone *et al.* (2013), Vahedi *et al.* (2014) and Greiman and Tkach (2012) for nematodes, trematodes and cestodes respectively were used to identify the helminth parasites that may have been in the faecal samples of bats.

3.3.4.2 DNA extraction (Faecal samples)

Extraction of DNA was done with the Omega bio-tek E.Z.N.A. Stool DNA Kit. 200mg of glass beads was put in a 2ml microcentrifuge tube and 200µl of the stool sample added.

540µl of the SLB Buffer was added and the mixture vortexed at maximum speed for 10 minutes. 60µl of DS Buffer and 20µl of Proteinase K Solution were added and the mixture vortexed. This was then incubated at 70^0C for 10 minutes. The sample was occasionally vortexed while it incubated. 200µl of SP2 Buffer was added to the mixture and the sample vortexed again at maximum speed for 30 seconds. The tubes containing the samples were then placed in ice and left to sit for 5 minutes.

The tubes containing the samples were centrifuged at full speed (≥ 13,000xg) for 5 minutes. 400µl of the supernatant was pipetted into a new 1.5 ml microcentrifuge tube being careful not to disturb the pellet or transfer any debris. 200µl of HTR Reagent was then added to the supernatant in the new tube and vortexed at maximum speed for 10 seconds. The mixture was left to sit at room temperature for 2 minutes and centrifuged at maximum speed for 2 minutes. 250µl of BL Buffer and 250µl of 100% ethanol were added and the mixture vortexed at maximum speed for 10 seconds. A HiBind® DNA Mini Column was then inserted into a 2ml collection tube. The entire lysate was transferred into the column including any precipitates that may have formed to the HiBind® DNA Mini Column. This was centrifuged at maximum speed for 1 minute and the filtrate and collection tube discarded. The HiBind® DNA Mini Column was then transferred into a new 2ml collection tube and 500µl VHB Buffer added. This was centrifuged at maximum speed for 30 seconds and the filtrate discarded but this time reusing the collection tube. 700 µl of DNA Wash Buffer was added to the column and the tube centrifuged at maximum speed for 1 minute. The filtrate was discarded and the collection tube reused. Sample was washed again by adding 700µl of DNA Wash Buffer to the column and the tube centrifuged at maximum speed for 1 minute. The filtrate was discarded and the collection tube reused. The column

and the collection tube were centrifuged at maximum speed for 2 minutes to dry the column. The column was then transferred into a clean 1.5ml microcentrifuge tube and 200µl of Elution Buffer, preheated to 65^0C, added directly to the center of the HiBind® matrix. The sample was then left to sit at room temperature for 2 minutes and the tube centrifuged at maximum speed for 1 minute. The DNA extracted was then stored at -20^0C. A NanoDrop was used to determine if the samples contained any DNA before PCR was done.

3.3.4.3 Polymerase Chain Reaction (PCR) (Faecal Samples)

Table 3.2: Reagents in PCR mix for helminths.

A polymerase chain reaction was performed by adding the following in the respective volumes. Details of forward and reverse primers are as shown in table 3.3.

Reagents in PCR mix	**Volume/ µl**		
	Nematodes	**Trematodes**	**Cestodes**
Maxima Hot Start PCR Master Mix (2x)	25	25	25
Forward Primer	0.5	0.5	0.5
Reverse Primer	0.5	0.5	0.5
Template DNA	10	10	10
Nucleus-free Water	14	14	14
Total	50	50	50

PCR for nematodes was performed using the following cycling condition: 95^{0}C for 15 min; 40 cycles of 94^{0}C for 30 s, annealing at 53◦C for 30s, 72^{0}C for 1 min; final elongation at72^{0}C for 10 min.

PCR for cestodes was performed using the following cycling condition: 5 min denaturation hold at 95^{0}C; 40 cycles of 30s at 94^{0}C, 30s at 52-56^{0}C, 2 min at 72^{0}C; and 7 min extension hold at 72^{0}C.

PCR for trematodes was performed using the following cycling condition: Denaturation at 94^{0}C for 5 min; 35 cycles of 94^{0}C for 60s, 54^{0}C for 50s and 72^{0}C for 80s; and final extension at 72^{0}C for 7 min.

3.3.4.4 Agarose Gel Electrophoresis (Faecal Samples)

Two percent of agarose gel was prepared by putting 1.5g of agarose into 100 ml of 1x TAE buffer into a conical flask. The mixture was heated until almost boiling and the agarose well dissolved. 2µl of ethidium bromide (EtBr) was added to the solution. Ethidium bromide binds to the DNA and allows one to visualize the DNA bands under ultraviolet (UV) light. The agarose gel mixture was then allowed to cool for a bit and then poured into a gel tray with the comb well in place. This was allowed to sit at room temperature for about 20 to 30 minutes until it was completely solidified. After the gel had solidified, the well comb was taken out carefully so the wells were well set. Once solidified, the agarose gel was placed into the gel box (electrophoresis unit). The gel box was then filled with 1x TAE until the gel was completely covered.

A molecular weight ladder was carefully loaded into the first well in the gel. 5µl of loading buffer was added to 10µl of each sample and carefully loaded into different well in the gel.

The gel was then run at 100 volts until the dye line is approximately 75 to 80 percent of the way down the gel. The power was then turned off, the electrodes disconnected from the power source and the gel carefully removed from the gel box. An Ultra Violet viewing machine was used to visualize the DNA fragments and photographs of the gel taken using a Gel Doc system.

3.4 Ethical approval and licensing

All procedures undertaken in the course of this thesis were approved by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research.

3.5 Statistical analysis

Data collected from the field was entered into an Excel spreadsheet. The statistical package "R" (R Core Team, 2014) was used for analysis by importing the data from Excel. A Shapiro-Wilk test was used to check for normality of the data which proved the data not to be normally distributed therefore non-parametric tests were used in the analysis of data. Pearson's Chi-squared test was used to test for significance in parasitic load among the age categories of bats and also the species of bats because the data was unpaired and mutually exclusive. The significance in parasite load between sexes of bats was however tested using the Mann-Whitney U test which is used to compare two population means or median that come from the same population. A Kruskal-Wallis H test was used to test for significance in infestation load among the capture sites and also significance in haemoparasite infection in bats. A Kruskall-Wallis H test was used because samples were more than two and independent of each other which makes it the most appropriate test to use.

Table 3.3: PCR primers used in amplification of extracted DNA.

Target group	Primer name	Sequence (5'-3')	Barcode	Amplicon length (bp)	Purpose
Plasmodium species	DW2	TAATGCCTAGACGTATTCCTGATTATCCAG	C310A	30	Genus
	DW4	TGTTTGCTTGGGAGCTGTAATCATAATGTG	C310B	30	
Nematodes	18S 965	GGCGATCAGATACCGCCCTAGTT	C310E	23	Family
	18S 1573	TACAAAGGGCAGGGACGTAAT	C310F	21	
Trematodes	ITS1 BD1	GTCGTAACAAGGTTTCCGTA	C3112	20	Family
	ITS1 4S	TCTAGATGCGTTCGAA(G/A)TGTCGATG	C3113	25	
Cestodes	cestl2	AAGCATATCAATAAGCGG	C3114	18	Family
	1200r	GCATAGTTCACCATCTTTCGG	C3115	21	

Primers for plasmodium were obtained from Schaer *et al*., 2013; Nematodes from Guardone *et al*., 2013; Trematodes from Vahedi *et al*., 2014; Cestodes from Greiman and Tkach,2012.

CHAPTER FOUR

4.0 RESULTS

4.1 Bat population composition

A total of 480 individual bats belonging to eight genera and nine species were captured and examined. The species composition of bats examined is shown in Table 4.1. *Epomophorus gambianus* constituted the highest number (57.29%) while *Nanonycteris veldkampi* were the least (1.04%) captured. Overall, most of the bats (54.79%) were captured at Ve-Golokuati while the lowest number of bats were captured at the '37' Military Hospital in Accra. Details of the species composition of bats captured at the four study sites are presented in Table 4.1. The least species diversity was observed at the '37' Military Hospital site where a single *E. helvum* and 47 *E. gambianus* were captured. The highest number of bat species was recorded at Buoyem, where all the species of bats recorded in this study occurred. *Rousettus aegyptiacus, E. franqueti, M. pusillus* and *N. veldkampi* were not recorded at the Tanoboase Sacred Grove. Although Ve-Golokuati had the highest total number of individual bats captures, three of the species recorded in the study; *R. aegyptiacus L. angolensis* and *H. monstrosus* were not found at this site (Table 4.1).

Table 4.1: Numbers and species composition of examined bats at each of the four capture sites.

Species	Total numbers at capture site (%)				
	37' Military Hospital	Buoyem	TSG	Ve-Golokuati	**Total**
Epomophorus gambianus	47 (97.92)	36(31.30)	19 (35.19)	173 (65.78)	**275**
Rousettus aegyptiacus	0 (0.00)	35 (30.43)	0 (0.00)	0 (0.00)	**35**
Lissonycterus angolensis	0 (0.00)	14 (12.17)	3 (5.56)	0 (0.00)	**17**
Epomops buettikoferi	0 (0.00)	2 (1.74)	2 (3.70)	3 (1.14)	**7**
Epomops franqueti	0 (0.00)	6 (5.22)	0 (0.00)	15 (5.70)	**21**
Eidolon helvum	1 (2.08)	1 (0.87)	28 (51.85)	2 (0.76)	**32**
Hypsignathus monstrosus	0 (0.00)	1 (0.87)	2 (3.70)	0 (0.00)	**3**
Micropteropus pusillus	0 (0.00)	16 (13.91)	0 (0.00)	69 (26.24)	**85**
Nanonycteris veldkampi	0 (0.00)	4 (3.48)	0 (0.00)	1 (0.38)	**5**
Total	**48**	**115**	**54**	**263**	**480**

Male bats were more than female bats, recording 262 and 217 individual bats respectively (Table 4.2). Except for *E. buettikoferi* and *H. monstrosus* species which consisted of only females, all others had both males (54.58%) and females (45.21%).

Bats collected were sorted into three developmental categories; adults (38.33%), sub-adults (30.21%) and juveniles (31.04%) (Table 4.2). Adult bats were the most captured while sub-adult bats were the least captured. Since majority of the bats captured were of the species *E. gambianus*, they also constituted the major species in all the developmental categories. No sub-adult and juvenile bats of the species *E. buettikoferi*, *N. veldkampi* and *L. angolensis* were captured (Table 4.2).

Table 4.2: Sex and age categories of bat species captured from all the four study sites.

Species	Sex		Age		
	M	F	Adult	Sub-adult	Juvenile
Epomophorus gambianus	160	115	90	82	103
Rousettus aegyptiacus	24	11	15	12	8
Lissonycterus angolensis	12	5	13	4	0
Epomops buettikoferi	0	7	6	0	1
Epomops franqueti	10	11	6	3	12
Eidolon helvum	23	9	26	5	1
Hypsignathus monstrosus	0	3	1	1	1
Micropteropus pusillus	31	54	24	38	23
Nanonycteris veldkampi	1	4	5	0	0
Total	**262**	**217**	**186**	**145**	**149**

4.2 Bat parasite species composition

4.2.1 Ectoparasites

Ectoparasites belonging to 27 different species were collected from the bats examined in this study. These comprised of 136 bat flies (Table 4.3), 157 mites (Table 4.5), 355 ticks (Table 4.6) and a single bat bug. The ectoparasite species found on the bats belonged to the following families: Nycteribiidae (Plate 4.1); Spinturnicidae, Carpoglyphidae, Psoroptidae, Tyroglyphidae, Trombiculidae, Pyemotidae (Plate 4.2); Argasidae and Ixodidae (Plate 4.3).

The predominant species of bat fly was *Nycteribia alternata* which occurred on five species of bats; *Epomophorus gambianus, R. aegyptiacus, L. angolensis, E. helvum* and *N. veldkampi.* Of the 480 bats examined, 7.71% (36) were found to harbour *N. alternata*. The second most abundant bat fly recorded was *Cyclopodia greefi greefi* which occurred on four species of bat (*E. gambianus, R. aegyptiacus, L. angolensis* and *E. helvum*) (Table 4.3). *Eucampsipoda africanum* was recorded on only two bat speces, *R. aegyptiacus* and *L. angolensis,* while *Eremoctenia vandeuseni,* was recorded only on *L. angolensis*.

Lissonycteris angolensis harboured four species of bat flies (Table 4.3), making it the bat species with the most numerous bat fly species. Bat flies were not recorded on the bat species *E. buettikoferi, E. franqueti, H. monstrosus* and *M. pusillus* (Table 4.3).

Table 4.3: Numbers of bat flies species collected from the different species of bats.

Bat species	Bat flies			
	Nycteribia alternata	*Eucampsipoda africanum*	*Cyclopodia greefi greefi*	*Eremoctenia vandeuseni*
Epomophorus gambianus	5	0	3	0
Rousettus aegyptiacus	26	5	11	0
Lissonycterus angolensis	17	4	8	4
Epomops buettikoferi	0	0	0	0
Epomops franqueti	0	0	0	0
Eidolon helvum	30	0	21	0
Hypsignathus monstrosus	0	0	0	0
Micropteropus pusillus	0	0	0	0
Nanonycteris veldkampi	2	0	0	0
Total	**80**	**9**	**43**	**4**

The highest number of bat flies (83) were collected from bats captured at Buoyem, followed by the Tanoboase Sacred Grove (43) and Ve-Golokuati (9) (Table 4.4). The only bat fly recorded at '37' Military Hospital was *N. alternata*. Bat fly species, *E. africana* and *E. vandeuseni* were not found at the Tanoboase Sacred Grove and Ve-Golokuati.

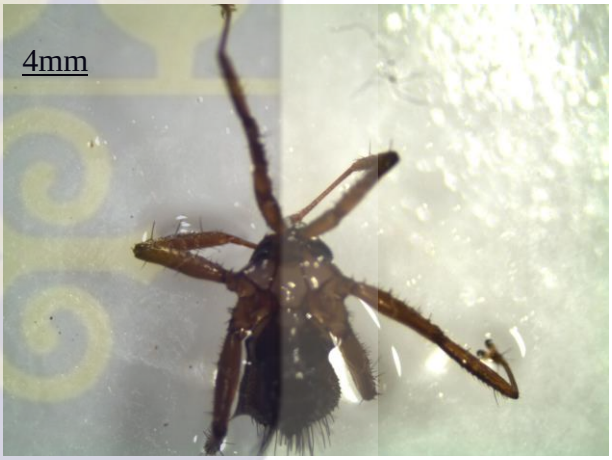
Table 4.4: Species and numbers of bat flies recorded on bats captured at the four study sites.

	Capture site			
Bat fly species	37' Military Hospital	Buoyem	Tanoboase Sacred Grove	Ve-Golokuati
Nycteribia alternata	1	51	23	5
Eucampsipoda africanum	0	9	0	0
Cyclopodia greefi greefi	0	19	20	4
Euremoctenia vandeuseni	0	4	0	0
Total	**1**	**83**	**43**	**9**

A – *Eucampsipoda africanum*

B – *Cyclopodia greefi greefi*

C – *Nycteribia alternata*



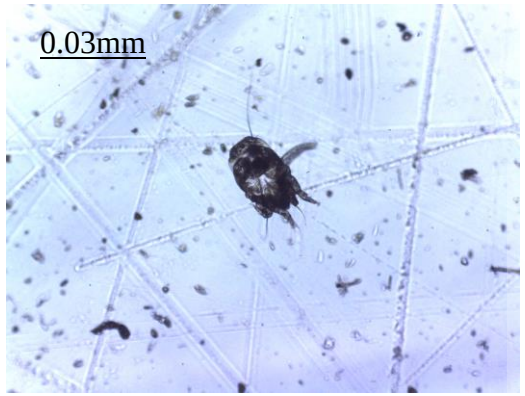
D- *Eremoctenia vandeuseni*

Plate 4.1: Pictures of bat flies found to infest bats examined during the study; A)- *Eucampsipoda africanum*; B)- *Cyclopodia greefi greefi*; C)- *Nycteribia alternata* and D)- *Eremoctenia vandeuseni.*

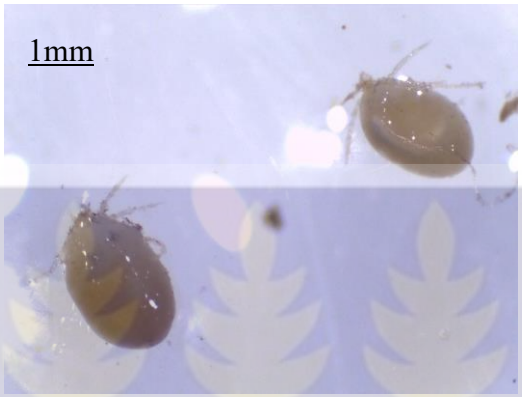
Eighteen different identified species of mites were recorded on the bats examined (Table 4.5). The mite species *Carpoglyphus* sp. and *Spinturnix* sp. were the most common, occurring on six different species of bats. Eight species of mites occurred on only one species of bat; *S. verutus* (only on *E. helvum*), *P. paracutisternus* and *P. ojastii* (*R. aegyptiacus*), *P. grandisoma* and *P. gameroi* (*E. gambianus), P. ridis* (*M. pusillus*) and *Trombicula* sp. (*N. veldkampi*) (Table 4.5) while *Rousettus aegyptiacus* harboured as many as ten different mite species. Other species of bats eg *Epomops buettikoferi* and *Nanonycteris veldkampi* hosted only one species of mite each.

Table 4.5: Mites on different species of bats examined.

	Numbers collected on bat species									
Mite species	*E. gambianus*	*R. aegyptiacus*	*L. angolensis*	*E. buettikoferi*	*E. franqueti*	*E. helvum*	*H. monstrosus*	*M. pusillus*	*N. veldkampi*	**Total no. of bat hosts**
Spinturnix americana	1	2	0	0	0	12	0	0	0	**3**
Spinturnix verutus	0	0	0	0	0	1	0	0	0	**1**
Steatonyssus longipes	0	3	0	0	0	0	0	0	0	**1**
Meristaspis kenyaensis	0	2	0	0	0	6	0	0	0	**2**
Ancystropus zeleborii	0	0	0	0	0	12	1	0	0	**2**
Spinturnix sp.	7	3	1	0	1	1	0	3	0	**6**
Acarus gracilis	5	0	0	0	1	0	0	0	0	**2**
Acarus sp.	4	2	0	0	1	0	0	0	0	**3**
Carpoglyphus sp.	21	3	2	0	2	4	0	6	0	**6**
Otodectes cynotis	1	0	0	0	2	1	0	5	0	**4**
Pomicronycte ridis	0	0	0	0	0	0	0	1	0	**1**
Pyemotis sp.	0	1	0	1	1	0	1	0	0	**4**
Chirptonyssus robustipes	3	1	0	0	0	1	0	2	0	**4**
Periglischrus paracutisternus	0	1	0	0	0	0	0	0	0	**1**
Periglischrus ojastii	0	1	0	0	0	0	0	0	0	**1**
Periglischrus grandisoma	1	0	0	0	0	0	0	0	0	**1**
Periglischrus gameroi	1	0	0	0	0	0	0	0	0	**1**
Trombicula sp.	0	0	0	0	0	0	0	0	25	**1**
Total number of individual mites collected	**44**	**19**	**3**	**1**	**8**	**38**	**2**	**17**	**25**	
Total no. of mite sp. harboured by each bat species	**9**	**10**	**2**	**1**	**6**	**8**	**2**	**5**	**1**	



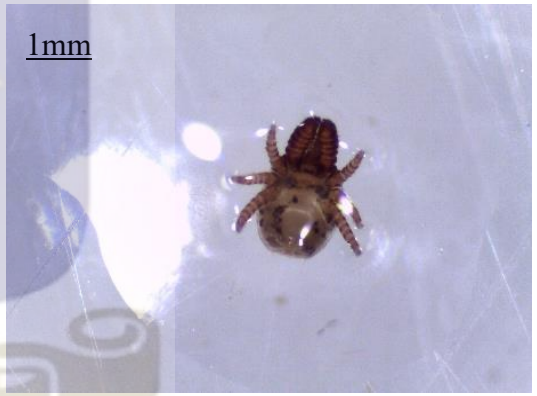
A- *Otodectes cynotis*



B- *Trombicula* sp.

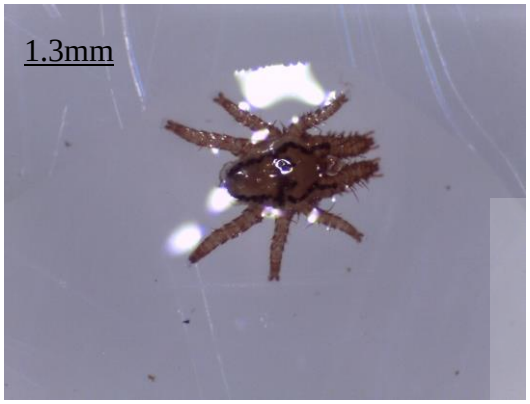
C- *Spinturnix verutus*

D- *Spinturnix americana*

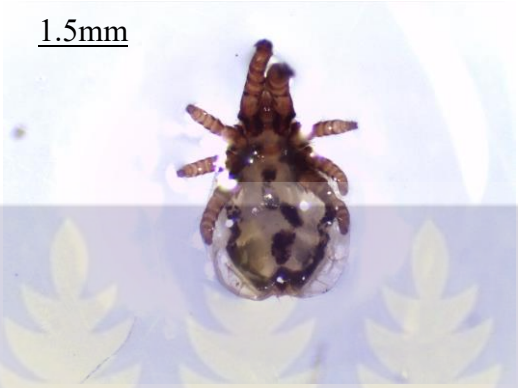


E- *Meristaspis kenyaensis*

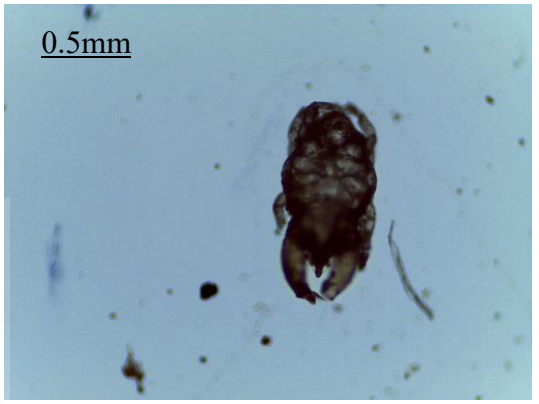
Plate 4.2: Mite species collected from captured bats from the study sites; A) - *Otodectes cynotis,* B) - *Trombicula* sp., C) - *Spinturnix verutus,* D) - *Spinturnix americana,* E) - *Meristaspis kenyaensis.*



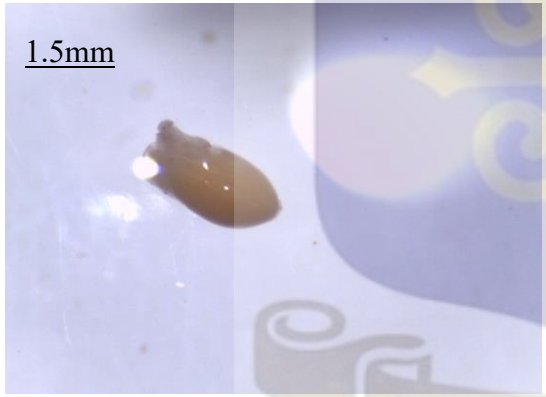
F- *Ancystropus zeleborii*



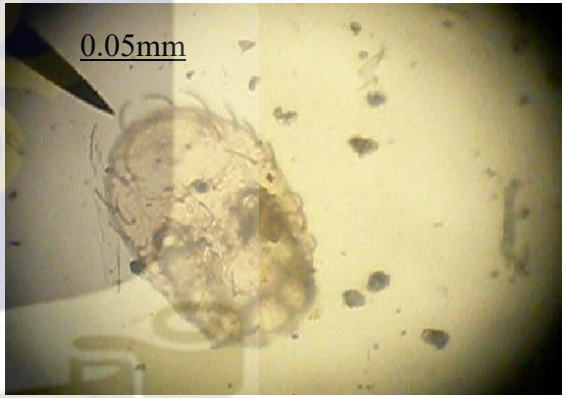
G- *Steatonyssus longipes*



H- *Acarus* sp.



I- Unidentified mite A



J- Unidentified mite B

Plate 4.2 continued: Mite species collected from captured bats from the study sites; F) - *Ancystropus zeleborii*, G) - *Steatonyssus longipes*, H) - *Acarus* sp., I) - Unidentified mite A, J) - Unidentified mite B.

In addition to the mite species reported above, two unknown ectoparasites were collected. These were suspected to be mites but are yet to be identified and have been labelled as mite species 'A' and mite species 'B' (Plate 4.2 continued, I and J) . For reasons unknown, majority of mite species 'A' were collected from *M. pusillus* and *E. gambianus* from Ve-Golokuati, but it also occurred on *R. aegyptiacus*, *L. angolensis* and *N. veldkampi* (Figure 4.1). About 29 percent of bats captured in Ve-Golokuati were parasitized with these unknown ectoparasite. The parasites were mostly cemented to the lower margin of the wings of the bats in great numbers and easily taken off the wing when ethanol was rubbed on them. They were attached so closely to the wing membrane of the bats, probably the mouthparts embeded in the skin, that most dislogded mites had lost their mouthparts making it difficult to identify these mite species. These mites may potentially be a new species yet to be named. *Epomops buettikoferi*, *E. franqueti*, *E. helvum* and *H. monstrosus* were not found to harbour this unknown mite species.

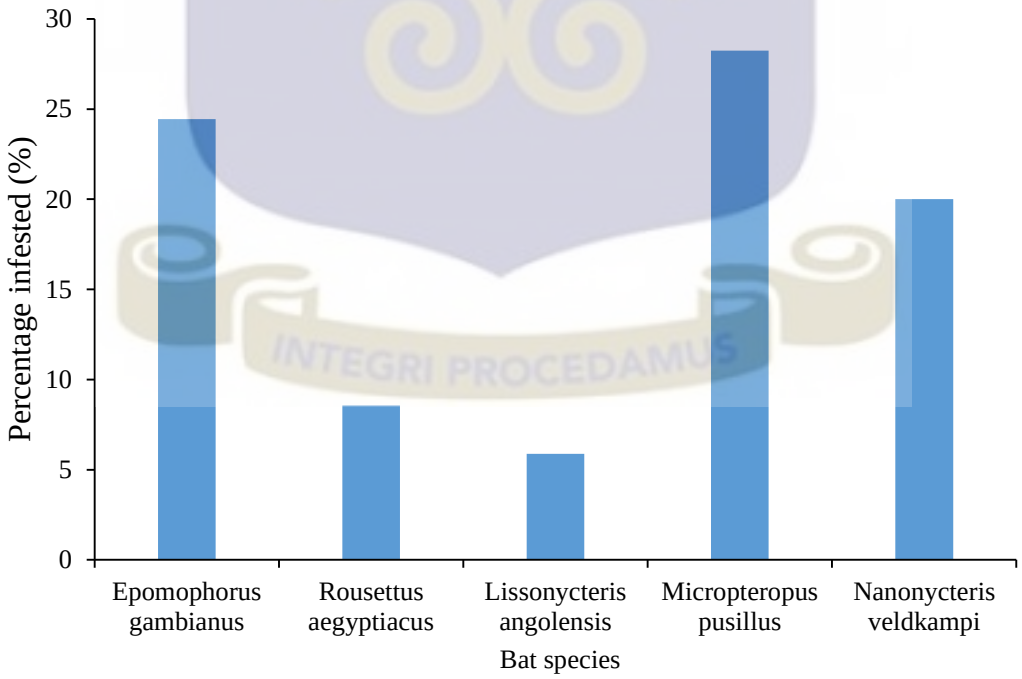


Figure 4.1: Prevalence of infestation of unknown mite species 'A' on the different bat species.

Mite species 'B' was recorded from five species of bats with the highest infestation occurring on the bat species *Epomops franqueti* (19.05%). *Epomops buettikoferi*, *E. helvum*, *H. monstrosus* and *N. veldkampi* did not harbour this mite species.

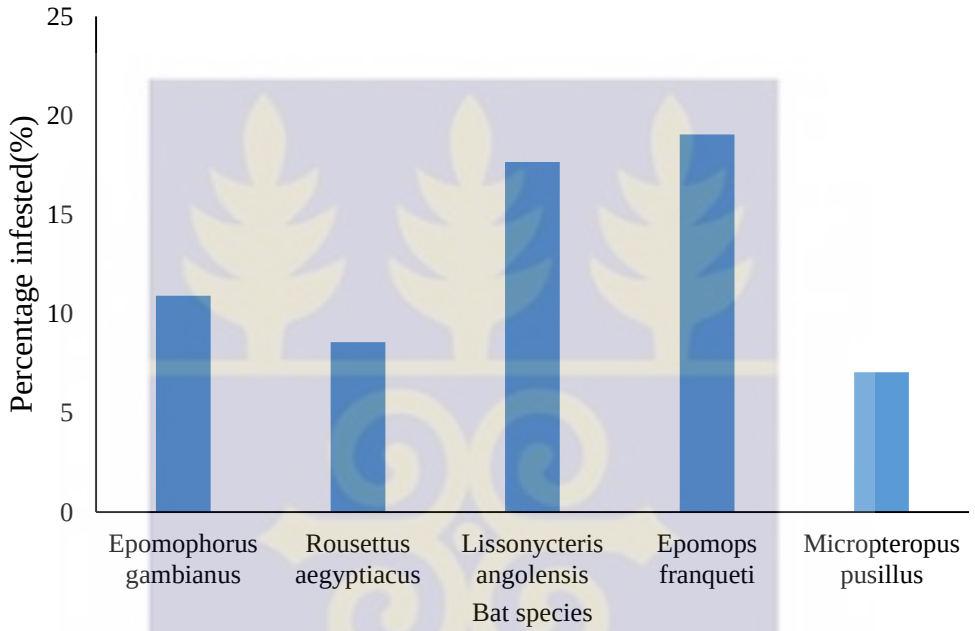


Figure 4.2: Prevalence of unknown mite species 'B' on the different bat species.

The tick species *Argas vespertilionis* was collected from three species of bats with the highest numbers occurring on *E. gambianus* (Table 4.6). A single individual of the larva an *Ixodes* sp. was collected from *R. aegyptiacus.*. The bat species *L. angolensis, E. buettikoferi, E. franqueti, E. helvum, H. monstrosus* and *N. veldkampi* did not harbour any ticks.

An individual *Leptocimex boueti* was collected on *E. gambianus.*

Table 4.6: Numbers and species of ticks collected from five species of bats.

	Bat ticks	
Bat species	*Argas vespertilionis*	*Ixodes* sp.
Epomophorus gambianus	352	0
Rousettus aegyptiacus	1	1
Lissonycterus angolensis	0	0
Epomops buettikoferi	0	0
Epomops franqueti	0	0
Eidolon helvum	0	0
Hypsignathus monstrosus	0	0
Micropteropus pusillus	1	0
Nanonycteris veldkampi	0	0
Total	**354**	**1**

Plate 4.3: A larva of an *Ixodes* sp. collected from a bat.

4.2.1.1 Prevalence of parasitic infestation

Of the 480 bats captured, 33.13% were infested with ectoparasites (Table 4.7). Although the most common bat species captured was *E. gambianus*, only a small proportion (1.8%) was infested with bat flies. Bat flies were dominant on *R. aegyptiacus* (57.14%), *L. angolensis* (70.59%) and *E. helvum* (56.25%).

All species of bats were infested with mites, the major ectoparasite found on bats (Table 4.7). The highest proportion of bats infested with mites was recorded in *Hypsignathus monstrosus* (66.67%). In all the other species of bats, less than 50% of the individuals examined were infested with mites. Tick infestation was generally lower than bat fly and mite infestation (Table 4.7)

Table 4.7: Ectoparasite (bat fly, mite and tick) infestation prevalence among bat species examined from all study sites.

Bat species	Total no. examined	Infestation with bat flies		Infestation with mites		Infestation with ticks	
		Infested (%)	Not infested (%)	Infested (%)	Not infested (%)	Infestation (%)	Not infested (%)
Epomophorus gambianus	275	1.80	98.20	15.27	84.73	6.91	93.09
Rousettus aegyptiacus	35	57.14	42.86	25.71	74.29	14.29	85.71
Lissonycterus angolensis	17	70.59	29.41	17.65	82.35	17.65	82.35
Epomops buettikoferi	7	0.00	100.00	14.29	85.71	0.00	100.00
Epomops franqueti	21	0.00	100.00	38.10	61.90	19.05	80.95
Eidolon helvum	32	56.25	43.75	43.75	56.25	0.00	100.00
Hypsignathus monstrosus	3	0.00	100.00	66.67	33.33	0.00	100.00
Micropteropus pusillus	85	0.00	100.00	17.65	82.35	5.88	94.12
Nanonycteris veldkampi	5	20.00	80.00	20.00	80.00	0.00	100.00

4.2.1.2 Parasite load

Of the 354 individual ticks collected from all bat species, 352 were collected from a single bat species (*E. gambianus*) giving a tick parasitic load of 1.28 ticks per bat in this species. The remaining three ticks came from *R. aegyptiacus* (2) and *M. pusillus* (1) (Table 4.8).

Although *E. gambianus* were heavily infested with ticks (1.28 ticks per bat), they were the least infested with bat flies. The number of bat flies recorded on *E. gambianus* ranged from 1 - 4 parasites per bat compared to 1 – 7 bat flies per bat in the other bat species. The average parasitic load of bat flies ranged from 0.03 in *E. gambianus* to 1.71 in *L. angolensis* (Table 4.8). There was a significant difference in bat fly infestation among the different species of bats with *L. angolensis* harbouring the heaviest load (X^2= 231.66, df = 8, p-value <0.01) (Table 4.8).

The heaviest infestation with mites occurred on *N. veldkampi* (5 mites per bat) (Table 4.8). *Epomops buettikoferi* were the least burdened with mites with a mean number of 0.14 per bat. The difference in mite infestation among species of bats was significant (X^2= 35.34, df = 8, p-value < 0.01).

Table 4.8: Parasite load on the different bat species.

	Bat flies		Mites		Ticks	
Bat species	Total no. of bat flies	Mean no./bat	Total no. of mites	Mean no./bat	Total no. of ticks	Mean no./bat
Epomophorus gambianus	8	0.03	43	0.16	352	1.28
Rousettus aegyptiacus	42	1.20	19	0.51	2	0.06
Lissonycterus angolensis	29	1.71	3	0.18	0	0.00
Epomops buettikoferi	0	0.00	1	0.14	0	0.00
Epomops franqueti	0	0.00	9	0.43	0	0.00
Eidolon helvum	50	1.56	42	1.13	0	0.00
Hypsignathus monstrosus	0	0.00	2	0.67	0	0.00
Micropteropus pusillus	0	0.00	16	0.19	1	0.01
Nanonycteris veldkampi	2	0.40	25	5.00	0	0.00

4.2.1.3 Influence of age of bat on ectoparasite infestation

The three groups of ectoparasites (bat flies, mites and ticks) were found to infest all age groups of bats that were captured. Among the three age groups (adults, sub-adult, juveniles), a higher proportion of adults harboured ectoparasites (56.52%) (Table 4.9). Comparatively however, a higher proportion of sub-adult bats were found to be infested with mites (30.34%) than the other groups (Adult= 77, Sub-adult= 48, Juvenile= 28) but the difference in infestation on the three age groups was not significant (X^2= 1.81, df = 2, p-value = 0.4051). The age group with the least proportion of individuals infested with ectoparasites was juveniles (Figure 4.2).

Adult bats carried the heaviest infestation of ticks; the number of ticks recorded ranged from 1 - 52 ticks with an average tick load of 0.69 per bat. There was no significant difference in tick infestation among age groups (X^2= 0.11, df = 2, p-value= 0.9455). The number of bat flies recorded on juveniles was one on all infested bats with an average tick load of 0.04 parasites per bat.

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Table 4.9: Prevalence and intensity of ectoparasite infestations (bat flies, mites and ticks) on different age classes of bats.

Age	Total no. of bats	Ectoparasite group	Total no. of bats infested	Proportion of bats infested (%)	Total no. of ectoparasites recorded	Mean no. of ectoparasite/bat
Adults	184	Bat flies	38	20.65	98	0.53
		Mites	51	27.72	77	0.42
		Ticks	15	8.15	127	0.69
Sub-adults	145	Bat flies	12	8.28	27	0.19
		Mites	44	30.34	48	0.33
		Ticks	12	8.28	99	0.68
Juveniles	149	Bat flies	6	4.03	6	0.04
		Mites	27	18.12	28	0.19
		Ticks	11	7.38	85	0.57

4.2.1.4 Influence of sex of bats on ectoparastie infestation

Except for male bats of the species *E. buettikoferi, H. monstrosus* and *N. veldkampi,* both sexes of all other species of bats were infested with ectoparasites. 100% of females of *R. aegyptiacus* and males of *E. helvum* were infested. Of the bat species infested with ectoparasites, a high proportion of *L. angolensis* male bats (83.33%) harboured ectoparasites while female bats of *E. gambianus* (13.91%) had the lowest proportion of infestation.

Of the five species of bats infested with bat flies, males of the species *L. angolensis* harboured the heaviest load of bat flies. The number of bat flies on individual male bats ranged from one to five with a mean of 2.33 parasites per bat (Table 4.10). Female bats of *E. gambianus* harboured the lowest load of bat flies, with number of bat flies on individual females ranging from 1 - 4 with an average parasite load of 0.01 per bat (Table 4.10). *Nanonycteris veldkampi* females harboured the highest load of mites while females of *E. gambianus* harboured the lowest load of mites per bats. The number of mites on individual females of *N. veldkampi* ranged from 1 – 25, with an average of 8.33 parasites per bat. No mites were recorded on males of *E. buettikoferi, H. monstrosus* and *N. veldkampi.* Mites were also absent on females of *L. angolensis.*

Males of *E. gambianus* harboured the highest load of ticks (Table 4.10). The total number of ticks on individual males of *E. gambianus* ranged from 1 to 88, with a mean of 1.52 ticks per bat. The lowest tick load was recorded on females of *M. pusillus* (Table 4.10). No ticks were recorded on males of *E. buettikoferi, E. helvum, H. monstrosus* and *N. veldkampi.*

A Mann-Whitney test was used to test for significance in infestation between the sexes of bats. The test revealed that infestation of male bats with bat flies was significantly higher than female bats (U= 26397, df = 1, p-value= 0.01584). There were however no significant difference

between the sexes in mite and tick infestations (U= 27829.5, df = 1, p-value= 0.5728 and U= 28116, df = 1, p-value= 0.6602 respectively).

Table 4.10: Sex differences in bat infestation with ectoparasites.

Bat species	Sex	Total no. of bats	Proportion of bats infested (%)	No. of bat flies/bat	No. of mites/bat	No. of ticks/bat
Epomophorus gambianus	M	160	22.50	0.04	0.18	1.52
	F	115	13.91	0.01	0.11	0.42
Rousettus aegyptiacus	M	24	87.50	1.25	0.5	0.50
	F	12	100.00	1.18	0.64	0.64
Lissonycteris angolensis	M	12	83.33	2.33	0.25	0.25
	F	4	25.00	1.25	0.00	0.00
Epomops buettikoferi	M	0	0.00	0.00	0.00	0.00
	F	7	0.00	0.00	0.14	0.00
Epomops franqueti	M	10	0.00	0.00	0.30	0.10
	F	11	0.00	000	0.55	0.27
Eidolon helvum	M	23	100.00	1.74	1.26	0.00
	F	9	88.89	1.44	1.33	0.00
Hypsignathus monstrosus	M	0	0.00	0.00	0.00	0.00
	F	3	66.67	0.00	0.67	0.00
Micropteropus pusillus	M	31	25.81	0.00	0.16	0.10
	F	54	27.78	0.00	0.22	0.06
Nanonycteris veldkampi	M	2	0.00	0.00	0.00	0.00
	F	3	66.67	0.67	8.33	0.00

4.2.1.5 Differences in ectoparasitic infestation of bats captured at the four different capture sites

4.2.1.5.1 Parasite prevalence in urban and rural sites

Grouping the capture sites into urban and rural areas, the highest proportion of bats infested with ectoparasites occurred at the rural areas (Tanoboase Sacred Grove, Buoyem, Ve-Golokuati) (30.32%) of total number of bats examined (Figure 4.3). The '37' Military Hospital, the only urban area, had a lower proportion of bats infested (22.92%) [11 out of 48].

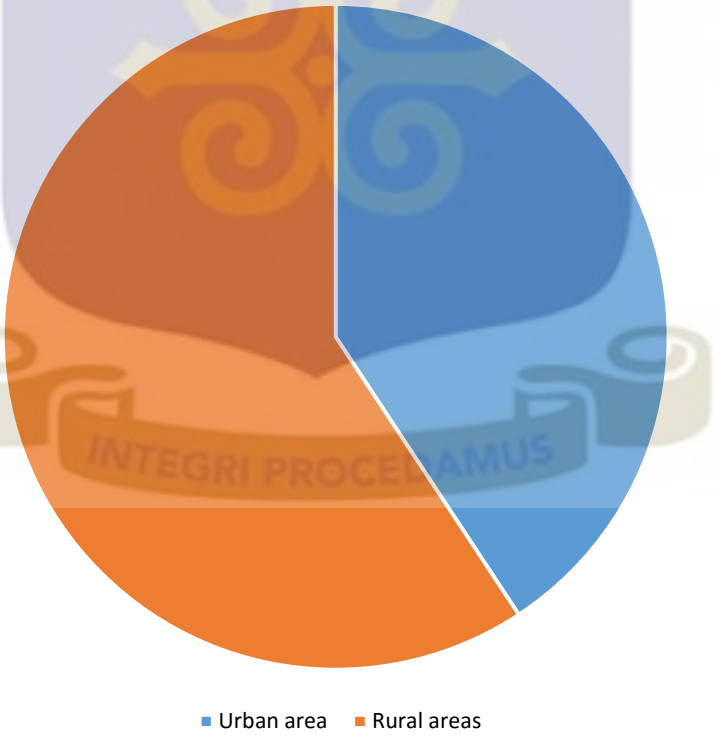

Figure 4.3: Proportion of ectoparasite infested bats in urban and rural communities.

The three major groups of ectoparasites (bat flies, mites, ticks) were recorded in all four study sites. The Tanoboase Sacred Grove site had the highest proportion of bats infested with ectoparasites (50%) while 26% and 29% of the bats captured at the Ve-Golokuati and Buoyem were infested with ectoparasites respectively.

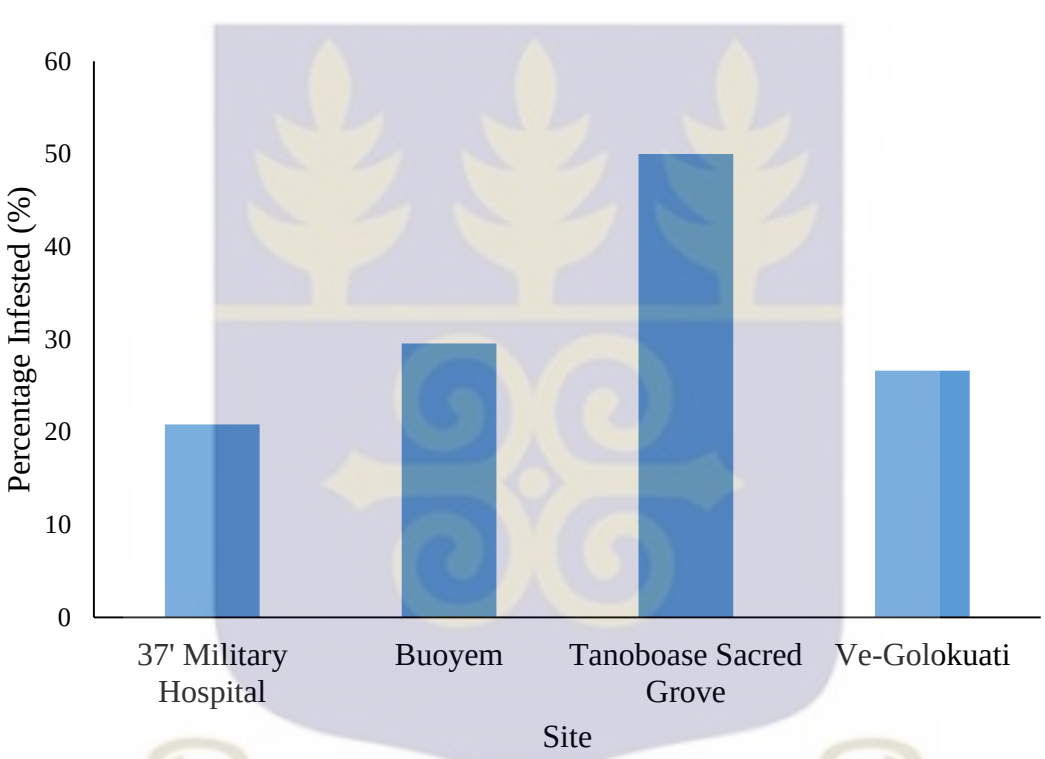

Figure 4.4: Comparison of the prevalence of ectoparasite infestation at the four capture sites.

4.2.1.5.2 Parasitic load of bats captured in urban and rural sites

Bats captured at the rural areas carried a higher ectoparasite load than bats captured at the urban areas (Figure 4.5). The number of bat flies recorded on individual bats in the rural areas ranged from one to six with an average of 0.24. Bats captured at the rural areas harboured mites ranging from 1 - 25 with an average of 0.40. Finally, the number of ticks recorded on individual bats in the rural areas ranged from 1 - 88 with an average of 0.66.

A Kruskal-Wallis H test showed a significant difference in bat fly infestation in rural and urban sites (X^2= 4.58, df = 1, p-value= 0.03233) with bats at the urban areas harbouring more ectoparasites per individual bat. There was no significant difference in load of mites and ticks infestations between urban and rural dwellings (X^2= 0.21, df = 1, p-value- 0.6441 and X^2= 2.51, df = 1, p-value= 0.1133 respectively).

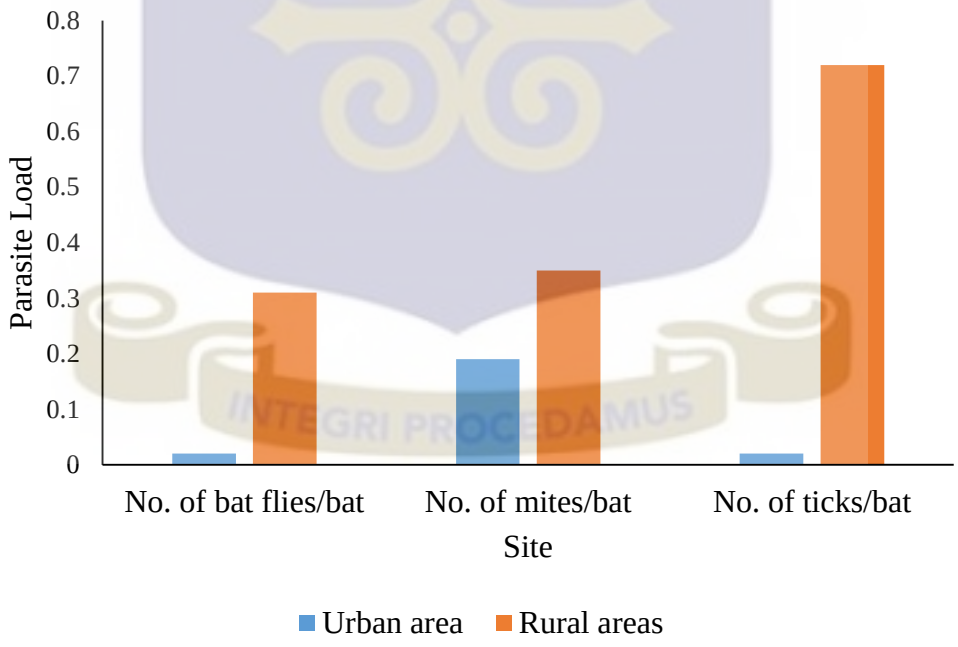

Figure 4.5: Comparison of the load of ectoparasite infestation on bats at urban and rural dwellings.

The highest load of bat flies (0.78) and mite (0.61) infestation per bat was harboured by bats captured at the Tanoboase Sacred Grove (Figure 4.5). In contrast, the lowest load of bat flies (0.02) and mites (0.19) per bat was harboured by bats at the '37' Military Hospital. Bats captured at Ve-Golokuati (Figure 4.6) harboured the highest load of ticks per bat (1.13) while the least infestation of ticks per bat occurred at the Tanoboase Sacred Grove (0.02).

A Kruskall-Wallis H test was used to test for significance in parasite load across the four study sites. Bats at the Tanoboase Sacred Grove harboured a significantly higher number of bat flies (X^2= 91.10, df = 3, p-value < 0.01) while '37' Military Hospital harboured the least load (Figure 4.6). On the contrary, the differences in load of mites and ticks were not significant (X^2= 6.83, df = 3, p-value= 0.0774 and X^2= 6.93, df = 3, p-value= 0.0742 respectively).

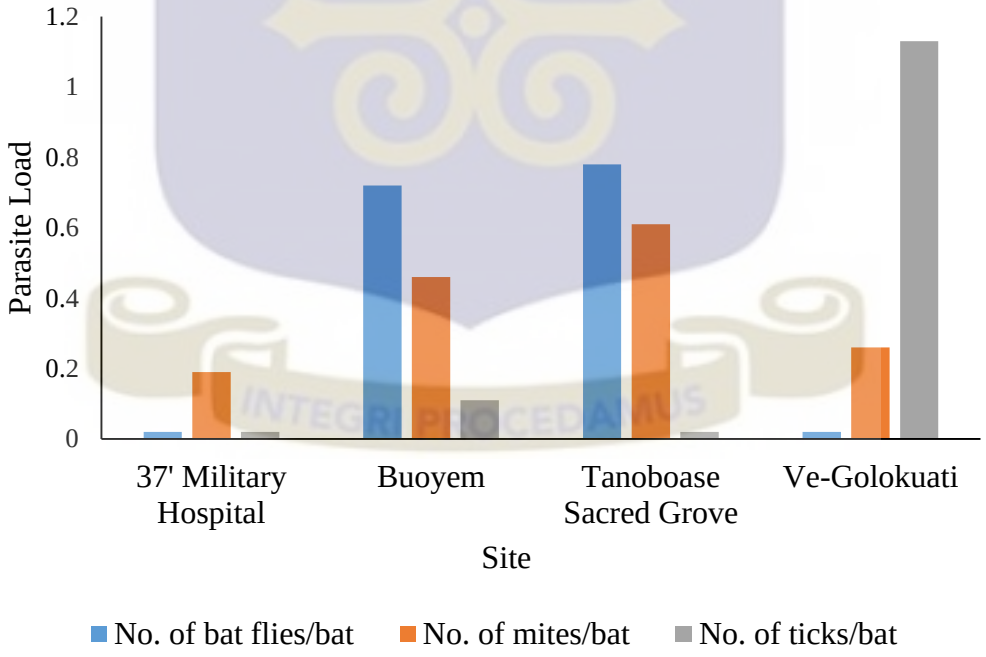

Figure 4.6: Comparison of load of bat fly, mite and tick infestations at the four capture sites.

4.2.1.6 Monthly variation in ectoparasite prevalence and intensity of infestation

4.2.1.6.1 Monthly variation in prevalence

The highest proportion of infested bats was recorded in the month of March (44.06%) while the lowest proportion was recorded in August (20%) (Figure 4.7).

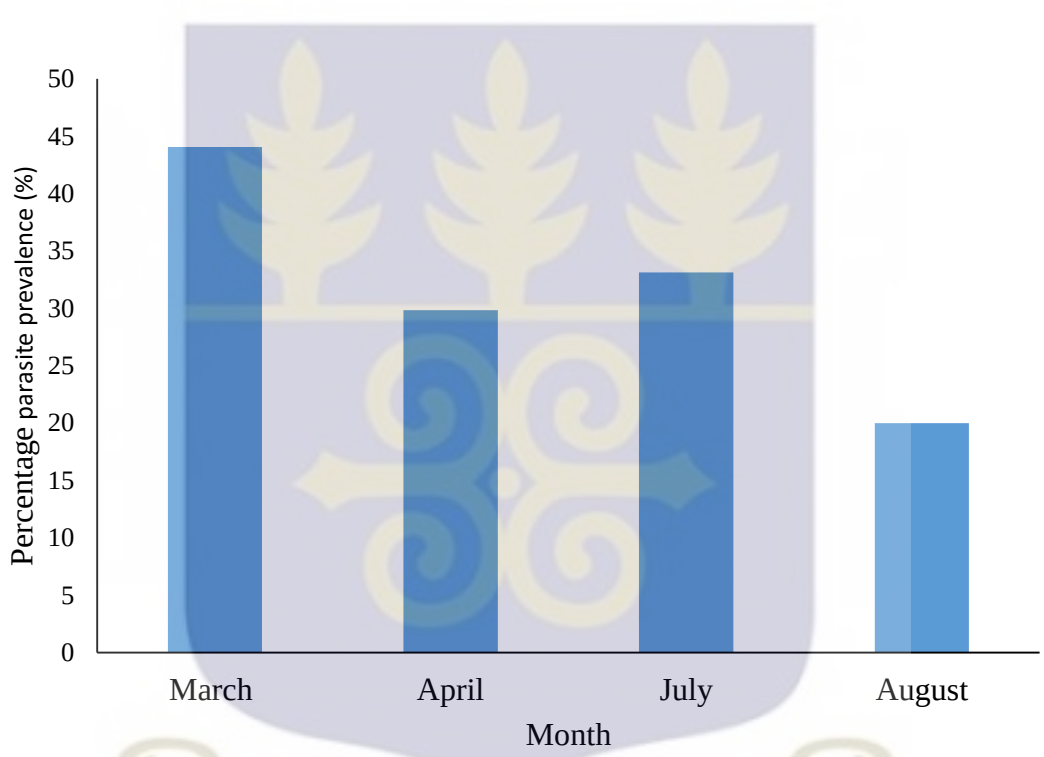

Figure 4.7: Monthly prevalence of ectoparasite on bats.

4.2.1.6.2 Monthly difference in ectoparasite load

Bats captured in March harboured the highest load of bat flies (0.53) than bats captured in the other months (April, July, August). No bat flies were recorded in April. The highest load of mites was recorded in July (0.41) and the lowest in April (0.09). The difference in bat fly and mite infestations among the months was significant (Kruskal-Wallis H test: X^2= 27.43, df = 3, p-value < 0.01 and Kruskal-Wallis H test: X^2= 8.28, df = 3, p-value= 0.04 respectively). In contrast, the highest number of ticks per bats occurred in the month of April (5.00) while the least number of ticks per bat was recorded in August (0.02) (Figure 4.8). There was also a significant difference in tick infestation among the months (Kruskal-Wallis H test: X^2= 24.76, df = 3, p-value < 0.01). For the three ectoparasite groups studied, their mean ectoparasite load varied significantly between the months.

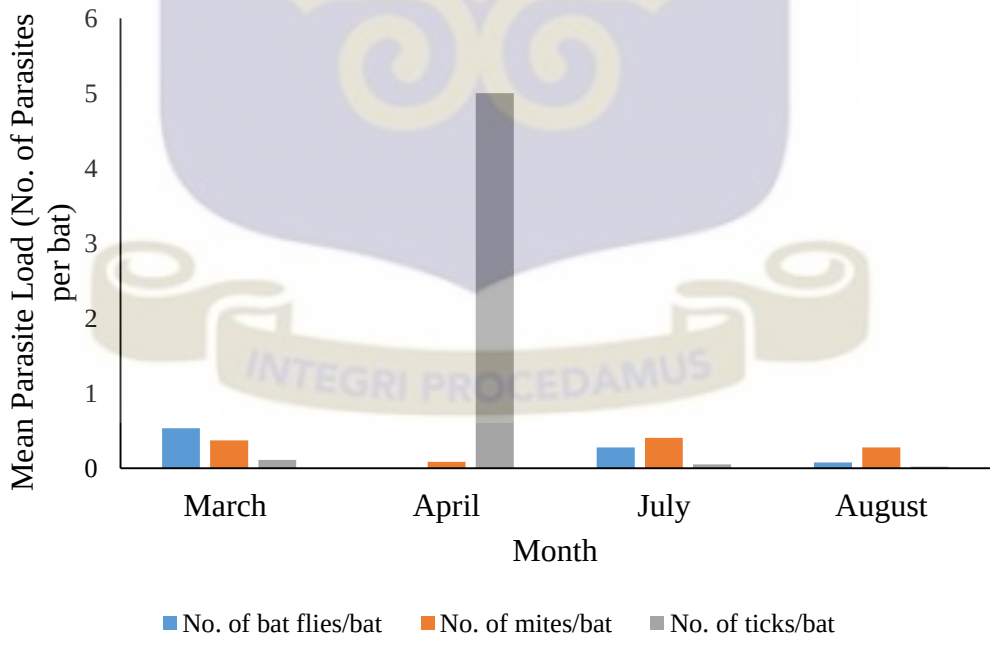

Figure 4.8: Monthly comparison of ectoparasitic load on total number of bats captured.

4.2.1.7 Influence of reproductive status on ectoparasite infestation of female bats

4.2.1.7.1 Differences in ectoparasite prevalence in reproductive and non-reproductive female bats

Female bats that were sampled from the various sites included pregnant and lactating females. *Epomophorus gambianus* had the highest proportion of pregnant female bats (31 out of 115) captured and *R. aegyptiacus* had the least number of pregnant females (1 out of 11) (Figure 4.11). Lactating females of *E. gambianus* captured (18 out of 115) were more than lactating females of the other species. No lactating females of *R. aegyptiacus, E. helvum* and *H. monstrosus* were captured.

All pregnant females of *R. aegyptiacus, E. helvum* and *H. monstrosus* were infested with ectoparasites. Pregnant females of *E. buettikoferi* and *E. franqueti* showed no infestation.

Table 4.11: Ectoparasite infestation prevalence of pregnant and lactating females examined from the different study sites.

Species	Total no. of females captured	No. of pregnant females	Proportion of pregnant females infested (%)	No. of lactating females	Proportion of lactating females infested (%)
Epomophorus gambianus	115	31	16.13	18	22.22
Rousettus aegyptiacus	11	1	100.00	0	00.00
Lissonycteris angolensis	5	2	50.00	1	00.00
Epomops buettikoferi	7	1	00.00	4	25.00
Epomops franqueti	11	2	00.00	2	50.00
Eidolon helvum	9	3	100.00	0	00.00
Hypsignathus monstrosus	3	1	100.00	0	00.00
Micropteropus pusillus	54	9	22.22	3	66.70
Nanonycteris veldkampi	4	4	75.00	1	100.00

4.2.1.7.2 Parasitic load of reproductive and non-reproductive females

The number of bat flies harboured by non-reproductive females ranged from 1-3 with a mean load of 0.15 bat flies per bat. The number of mites ranged from 1-5 with a mean of 0.34 mites per bat. Pregnant females harboured bat flies with numbers ranging from 1-5 with a mean of 0.26 bat flies per bat. The number of mites harboured by pregnant females ranged from 1 to 25 with a mean of 0.63 mites per bats. Lactating female bats harboured bat flies (0.07 bat flies per bat) and mites (0.24 mites per bat). The number of ticks on lactating females ranged from 1- 32 with a mean of 1.55 ticks per bats; there was no significant difference in tick infestation between lactating and non-reproductive females (Kruskal-Wallis H test: X^2 = 1.68, df = 2, p-value = 0.4317). Pregnant females did not harbour any ticks. There was no significant difference in bat fly (Kruskal-Wallis H test: X^2 = 0.84, df = 2, p-value = 0.6557) and mite (Kruskal-Wallis H test: X^2 = 1.58, df = 2, p-value = 0.4547) infestations between reproductive and non-reproductive females.

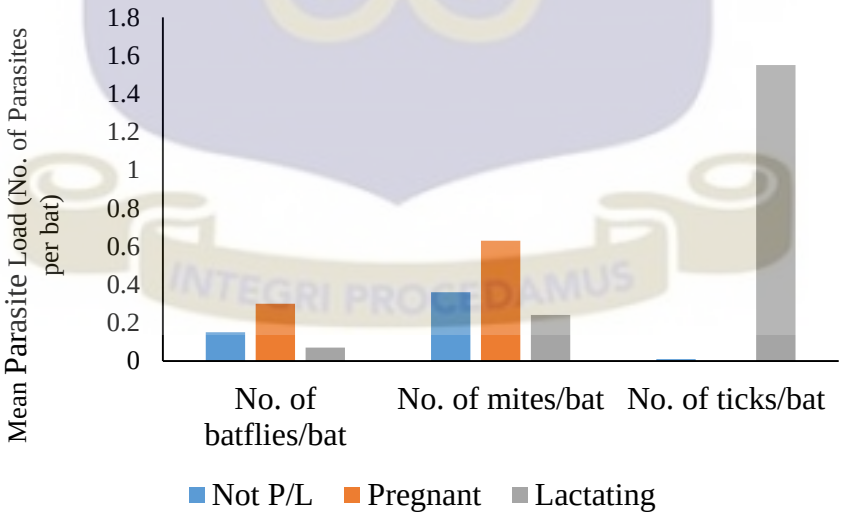

Figure 4.9: Comparison of load of bat fly, mite and tick infestations of female bats (non-reproductive, pregnant and lactating).

4.2.2 Bat infestation with endoparasites

4.2.2.1 Identification of haemoparasites

Approximately 48 percent of the total number of bats captured for which blood samples were taken and examined microscopically (Plate 4.4), were infected with haemoparasites. These haemoparasites were recorded in all the nine species of bats captured in the present study. Positive samples were molecularly tested to determine the species of haemoparasites. All samples tested negative, by PCR, for *Plasmodium* sp. (Plate 4.5), the species of interest. The control sample band remained visible after electrophoresis indicating that the PCR amplification conditions and reagent were ideal. Thus, the haemosporidian parasites observed in the blood of sampled bats by microscopy may not be of the genus *Plasmodium*. Other genera of haemosporidian parasites (*Nycteria* and *Hepatocystis*) known to infect fruit bats were not tested for due to logistical and time constraints. Further studies will be carried out later on to determine the species of haemosporidian parasites harboured by bats.

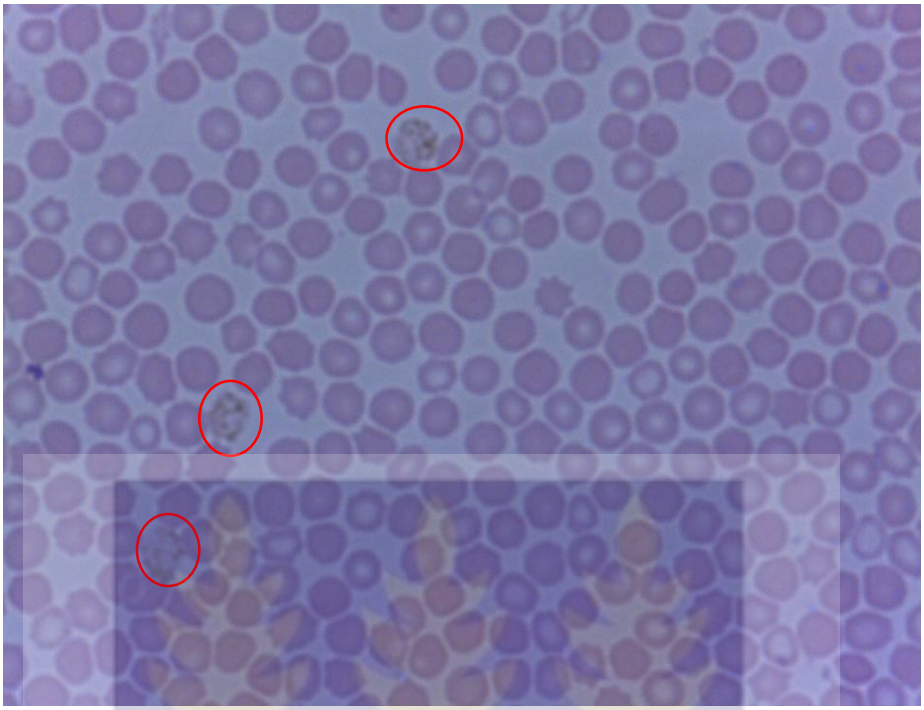

Plate 4.4: Slide showing bat blood cells infected with haemoparasites. Infected cells are depicted in the picture with red rings.

4.2.2.2 Prevalence of haemoparasite infection

The proportions of the different species of bats infected with haemoparasites are as shown in Figure 4.10. Prevalence of blood parasites infection was highest in *Nanonycteris veldkampi* (80%) with the lowest observed in *H. monstrosus* (33.33%).

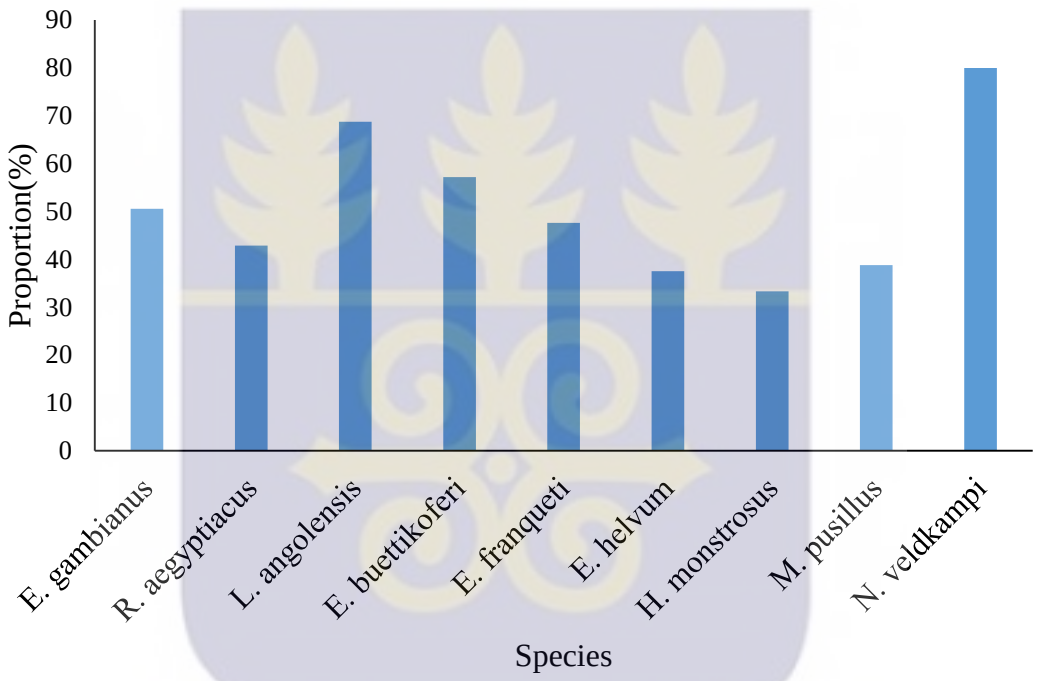

Figure 4.10: Percentage prevalence of haemoparasite infection among the nine species of bats examined during the study.

A higher proportion of male bats (48.85% [128 out of 262]) were infected with haemoparasites as compared to female bats (46.54% [101 out of 217]) (Figure 4.11), however the difference was not significant (Mann-Whitney test: U= 28335, df = 1, p-value = 0.9438). With respect to age categories, the highest prevalence of haemoparasitic infections occurred among juvenile bats (50.34% [75 out of 149] showed infection), with the least observed in sub-adult bats (45.52% (66 out of 145]) (Figure 4.12). There was again no significant difference in infestation among the age categories (Kruskal-Wallis H test: X^2= 0.26, df = 2, p-value = 0.8766).

Comparing infection prevalence in bats at the different capture sites, bats captured at the '37' Military Hospital showed the highest prevalence of 54.17% [26 out of 48] (Figure 4.13). In contrast, bats captured at Ve-Golokuati (43.35% [114 out of 263) were the least infected. There was no significant difference in infection of bats at the different capture sites (Kruskal-Wallis H test: X^2= 3.74, df = 3, p-value = 0.2911).

Comparing infection prevalence in bats in the different capture months, the highest proportion of infection was recorded in August (53.14%) while the least proportion was recorded in July (33.33%) (Figure 4.14).

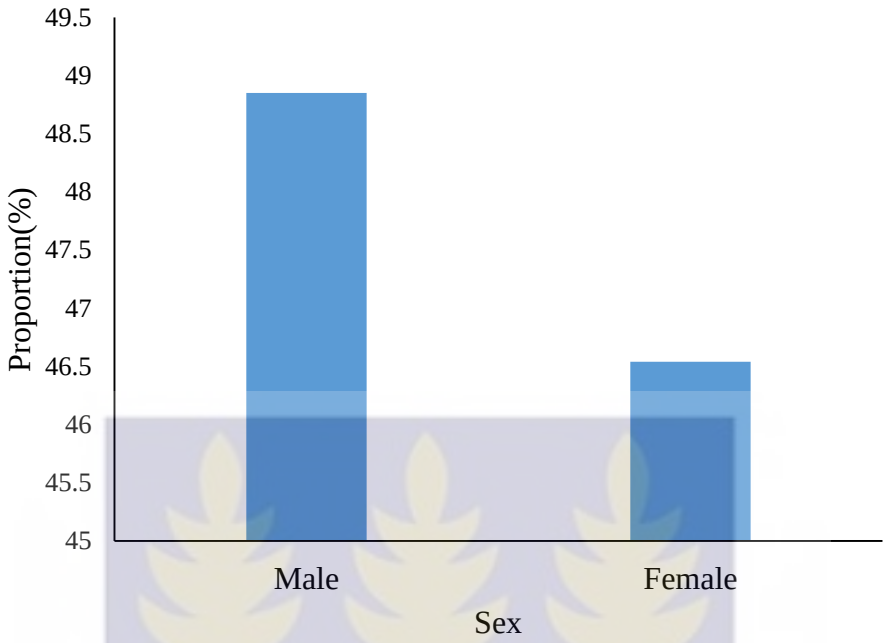

Figure 4.11: Proportion of male and female bats infected with haemoparasites.

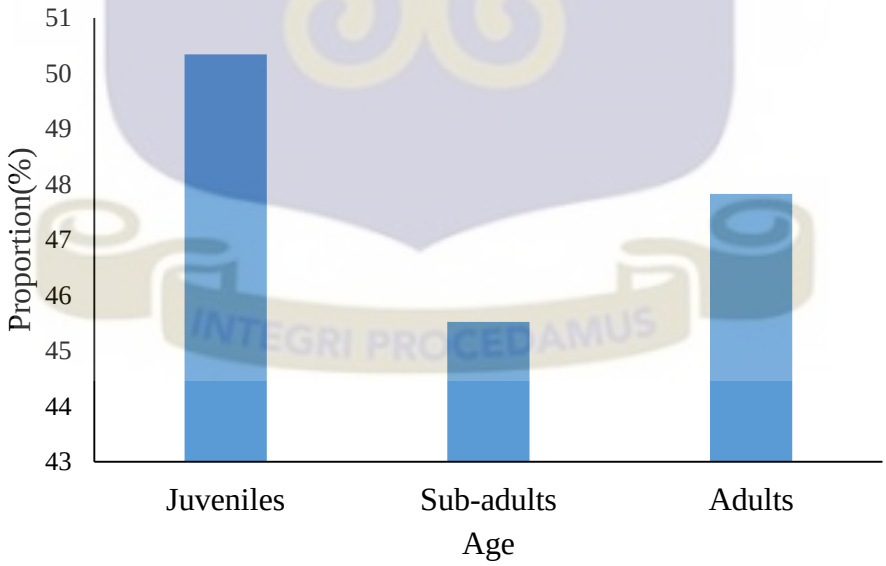

Figure 4.12: Prevalence of heamoparasitic infection among the different age categories of bats.

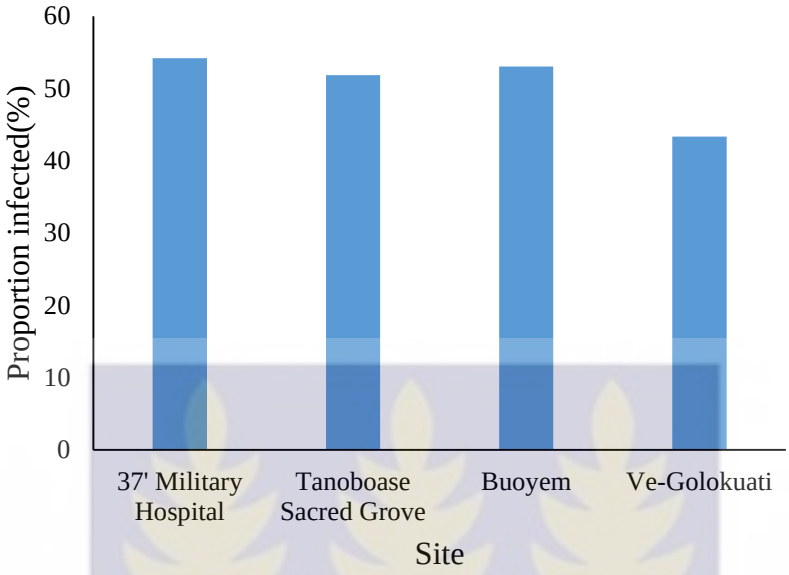

Figure 4.13: Prevalence of haemoparasitic infections among bats at the different capture sites.

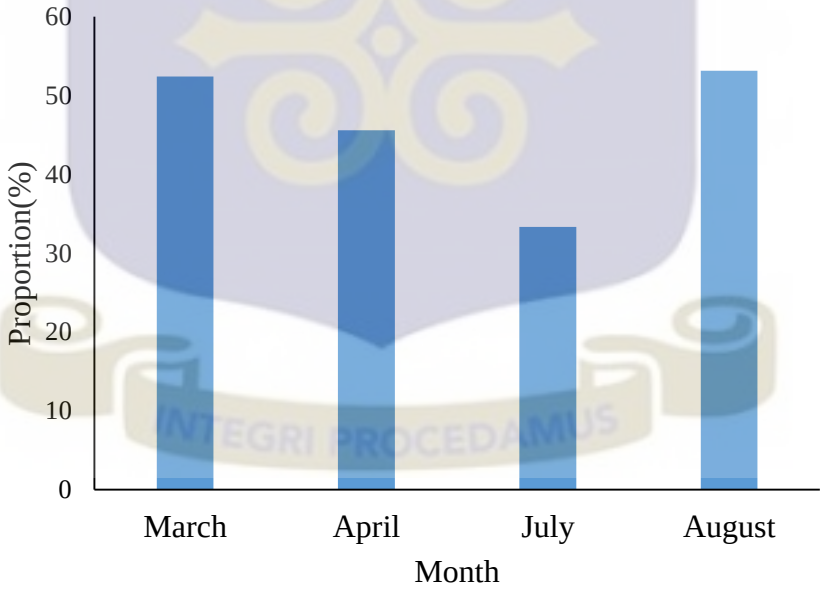

Figure 4.14: Proportion of bats infected with haemoparasites in the different months of capture.

The highest proportion of bats infected with haemoparasites were lactating female bats (68.97% [20 out of 29]) (Figure 4.15). Pregnant female bats were the least infected (42.59% [23 out of 54]). A Pearson Chi-Square test indicated that there was a significant difference in haemoparasite infection among female bats in the different reproductive status (X^2 = 37.738, df = 102, p-value < 0.01).

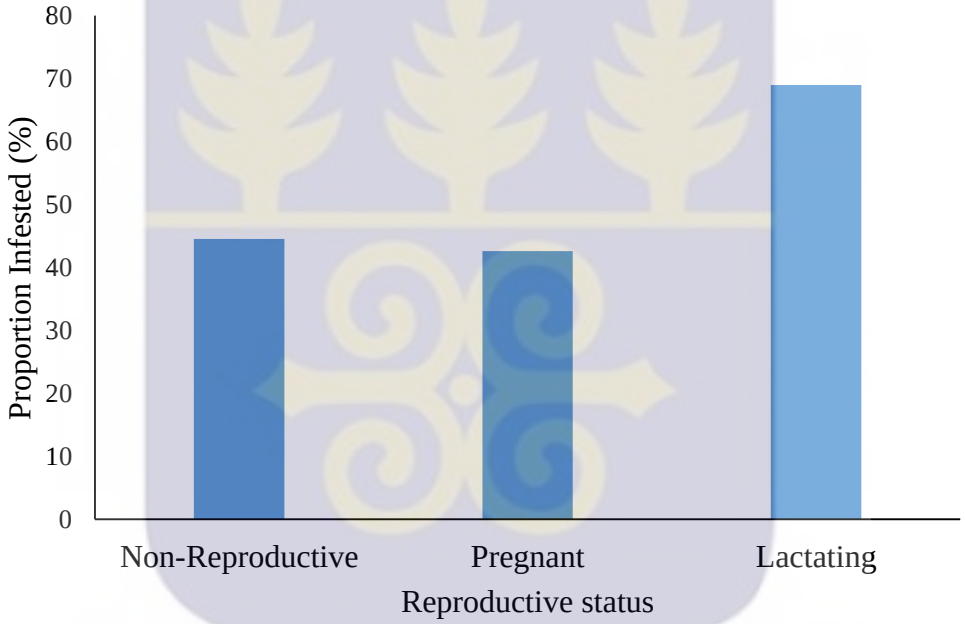

Figure 4.15: Proportion of female bats infected with haemoparasites.

4.2.2.3 Helminth Infestation

Faecal samples of 29 bats were screened by PCR for helminthes (nematodes, cestodes and trematodes). Positive and negative controls were used to assess the fidelity of the PCR reagents and thermal cycling conditions as well as serve as standards to compare with samples that were tested. Extracted DNA of *Schistosoma mansoni* and *Wuchereria bancrofti* were used for the controls for trematodes and nematodes respectively. *Taenia saginata* DNA was used as control for cestodes. All 29 samples tested negative.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Ectoparasite distribution and role in disease transmission

All species of bats captured and examined were infested with both ectoparasites (bat flies, ticks, mites, bat bug) and endoparasites (hemoparasites). The four species of bat flies recorded belonged to the superfamily Hippoboscoidea. This family is primarily found in the Old World tropics and only a few of the 274 known species occur in the Neotropics and in Europe (Graciolli and Dick, 2008). *Nycteribia alternata,* a common species of bat fly, was recorded on several of the bat species examined. These flies have been reported on bats from the Afadzato Agumatsa Range in a previous study (Nartey, Unpublished data). Maa (1962) indicated that this bat fly was first observed on *E. helvum* in Cameroun by Kerr in 1936. In this study, they were recorded on bats from the '37' Military Hospital, Buoyem and the Tanoboase Sacred Grove but not at Ve-Golokuati, which comes as a surprise considering the fact that Ve-Golokuati (N 06º 59.851′, E 000º 26.218′) is geographically not far from the Afadzato Agumatsa Range (N 7º 1.00', E 0º 34.00'). The bat species that harboured *N. alternata* were *Epomophorus gambianus*, *Rousettus aegyptiacus, Lissonycteris angolensis, Eidolon helvum* and *Nanonycteris veldkampi*. This confirms the fact that *N. alternata* is a common parasite of bats and is not host specific.

Cyclopodia greefi greefi, another common bat fly, has been reported to occur on *Rousettus angolensis* (now *Lissonycteris angolensis)* and *Epomophorus* sp. (Maa, 1962), but more commonly on *E. helvum*. In this study, *C. greefi greefi* was recorded on *R. aegyptiacus, E. gambianus* and *L. angolensis*, with the highest infestation occurring on *E. helvum* which confirms that, indeed, these bat fly species commonly occur on *E. helvum*. Funmilayo (1976)

also reported that *E. helvum* harboured higher numbers of *C. greefi greefi* than other species of bats in a study done in Nigeria. *C. greefi greefi* was also observed on *E. helvum* specimens from Ghana (Billeter *et al*., 2012). The occurrence of *C. greefi greefi* on *E. gambianus* is uncommon (ACR, 2014).

The bat fly *Eucampsipoda africanum* , on the other hand, was not as common as *N. alternata* and *C. greefi* greefi. Of the nine individual *E. africanum* recorded, five were picked from *R. aegyptiacus* and four from *L. angolensis*. *E. africanum* has been reported previously to occur commonly on these two species of bats as well as on *E. helvum* (Theodor, 1956). *E. africanum* is reported also to be widely distributed over the Ethiopian region and recorded from localities in Senegal to the Sudan and southwards to the Cape (Haeselbarth *et al*., 1966). Despite previous reports of *E. africanum* as a common bat fly of *E. helvum, R. aegyptiacus* and *L. angolensis* and the high numbers of these species of bats examined in the study, only four bats were found to be infested with this bat fly. The findings from this study suggests that the species may not be as common in Western Africa.

Eremoctenia vandeuseni was the most uncommon bat fly recorded in the study with just four individuals recorded on *Lissonycteris angolensis. E. vandeuseni* was first recorded on *Miniopterus* sp. from West New Guinea (Maa, 1962). According to Anciaux de Faveaux (1971), bat flies usually encountered on *Lissonycteris angolensis* include *Cyclopodia greeffi, Dipseliopoda biannulata* and *Dipseliopoda setosa.* However, none of these bat fly species were collected on *L. angolensis* in this study. The bat species *Epomops buettikoferi, Epomops franqueti, Micropteropus pusillus* and *Hypsignathus monstrosus* were not infested with bat flies and this supports the fact that no reported cases of infestation of the four bat species with bat

flies were found in the literature. These species of bats are known to roost singly or in small groups (Kunz, 1996; Jones, 1972; Bradbury, 1977) and this may limit cross infestation with bat flies common on other bat species. Incidentally, these bat species also rarely harboured mites and ticks.

Mites recorded on bats in this study belonged to the families Spinturnicidae, Carpoglyphidae, Psoroptidae, Tyroglyphidae, Trombiculidae and Pyemotidae. The highest incidence of mite infestation on bats occurred with mites belonging to the family Spinturnicidae which comprise three genera (*Spinturnix*, *Ancystropus* and *Meristaspis*). Mites of the family Spinturnicidae were recorded on all species of bats examined in this study.

Spinturnix sp. were the most common mites recorded and were found on six different bat species (*E.* gambianus, *R. aegyptiacus*, *E. helvum*, *H. monstrosus*, *L. angolensis* and *H. monstrosus*). According to Bruyndonckx *et al.* (2009), different species of the genus *Spinturnix* rarely occur on the same individual bat. However, in this study, an individual *Eidolon helvum*, was found to harbour three different *Spinturnix* species (*Spinturnix verutus*, *Spinturnix americana* and *Spinturnix* sp.). The mite species *Meristaspis kenyaensis* has been reported on *R. aegyptiacus* (Hafez *et al.*, 1994) and also on *E. helvum* (Dusbabek and Bergmans, 1980). In this study, *M. kenyaensis* was shown to infest *R. aegyptiacus* and *E. helvum* and may therefore be host specific to these species of bats (*E. helvum* and *R. aegyptiacus*). *Ancystropus zeleborii*, another common mite, was first reported from *R. aegyptiacus* by Kolenati (1857). In this study, *A. zeleborii* was collected from *E. helvum* and *H. monstrosus*. The only two species of bats from which mites were collected were *Epomops buettikoferi* and *Nanonycteris veldkampi*, probably due to the low number of these bat species captured, as suggested by Bertola *et al.* (2005). Not much is known

about ectoparasites infesting *E. buettikoferi* and *N. veldkampi* (ACR, 2014). However, a mite species *Ancystropus aethiopicus* has been recorded on *N. veldkampi* and *M. pusillus* (Hirst, 1923). In this study, *Trombicula* sp. (biting mites) were found on a single individual of *N. veldkampi.* This is a significant finding since *Trombicula* sp. have been reported to transmit *Orientia tsutsugamushi,* the causative agent of typhus fever in Asia and the Pacific region (Watt and Parola, 2003; Kelly *et al.,* 2009). Of significance also is the record of the Ebola virus reported in *N. veldkampi* species captured in Ghana (Hayman *et al.,* 2012). *N. veldkampi* thus remains an important bat species worth further investigation in order to shed light on their potential role as reservoirs of human pathogens.

Mites in general cause mainly severe allergic responses, eczema and asthma in humans causing discomfort and pain (Walker, 1996). However, *Spinturnix americana* and *Spinturnix verutus* have not yet been implicated in the transmission of any pathogens or diseases and therefore may not be of any public health importance immediately. *Otodectes cynotis* is found around the world (Hering,1838) and is known to occur in the ears of canids, foxes, cats, ferrets (Curtis, 2004; Otranto *et al.,* 2004). This mite was collected on four species of bats; *E. gambianus, E. franqueti, E. helvum* and *M. pusillus* in this study with the highest number occurring on *M. pusillus* (55.56%) and the least number occurring on *E. helvum* (11.11%) and *E. gambianus* (11.11%). *O. cynotis* feed on the epidermal debris inside the ear of the host and mainly causes *otitis externa,* which is associated with thick reddish-brown crusts in the ear canals. *O. cynotis* infection causes severe pruritis in these animals (Griffin *et al.,* 2001). It is possible for humans to acquire ear mites but this is rare. There are a few reports of suspected infections with Otodectes mites in people (Weese, 2010). One report cited ear mites on the torso and extremities of the owner of a cocker spaniel and another reported six mites from crusts on a woman's

eardrum (Curtis, 2004). *O. cynotis* infestation can lead also to secondary microbial infectons with bacteria and fungi (Roy *et al.*, 2011) hence they are of significant public health importance. Several *Acarus* sp. were recorded on the bats examined in the current study and were found on *R. aegyptiacus, E. franqueti* and most abundantly on *E. gambianus. Acarus* sp. affect humans in various ways. Human cases of enteric acariasis, caused by *Acarus* sp., have been reported where mites were found in excreta, suggesting their presence in the digestive tract (Martinez-Marañón and Hoffman, 1976). Most of the acarid mites implicated in these cases belong to the genera *Acarus*, *Suidasia*, or *Tyrophagus*. *Acarus* sp. could have significant effects on the health of humans in communities where they are endemic due to their role in causing enteric acariasis. *Suidasia ponsifica* was recorvered from the feaces of a woman and two infants in Mexico (Martinez- Marañón and Hoffman, 1976) while various stages of an *Acarus* sp. together with eggs, were recovered from the bile of a Romanian patient with chronic cholecystitis (Pitariu *et al.*, 1978).

Carpoglyphus sp. recorded on six bat species (*E. gambianus, R. aegyptiacus, L. angolensis, E. franqueti, E. helvum* and *M. pusillus*) in the current study, has been associated previously with a case of pulmonary acariasis (presence of mites in sputum) in Spain (Taboada, 1954). Pulmonary ascariasis causes allergies which could lead to severe allergic sensitization and discomfort in humans. *Carpoglyphus* sp. can cause also Grocer's itch, which is a cutaneous condition characterized by pruritic dermatitis (William *et al.*, 2006). The public health importance of other mite species recorded rarely in this study (*Periglischrus* sp., *Pyemotis* sp. and *Steatonyssus longipes*) is unknown.

Species belonging to two families of ticks (*Argas vespertilionis* and *Ixodes* sp.) were infected on *E. gambianus, R. aegyptiacus* and *M. pusillus. Argas vespertilionis* is a common tick

occurring on bats and has been reported to infest the bat species *R. aegyptiacus*, *Pipistrellus pipistrellus*, *Pipistrellus kuhlii* and *Tadarida teniotis* (Latreille, 1802). It would appear therefore that this tick species infest both fruit-eating and insect-eating bats. The rarity of *Ixodes* ticks on bats examined in this study suggests that ticks of the family Ixodidae do not commonly infest bats. Tick bites may cause severe allergic reactions and tick paralysis which in turn causes unsteady gait, weakness of the limbs, multiple rashes, headache, fever, flu-like symptoms, tender lymph nodes and partial facial paralysis (Traub and Cummins, 2011). Tick typhus caused by a bacterium, *Rickettsia* sp., which is transmitted by the tick *Amblyomma maculatum*, may also cause headaches, multiple rashes, swollen glands, fever and flu-like symptoms. The disease is rarely fatal and readily responds to antibiotic therapy (Webb *et al.*, 2012). *Borrelia* sp. is transmitted by the tick *Ixodes scapularis* and some common symptoms in humans infected include aches, fever, muscle and joint pain, and arthritis. According to Webb *et al.* (2012), twenty people died from tick paralysis between 1925 and 1945, but improvements in modern medicine and the development of a tick antitoxin have prevented further deaths in the last 65 years.

Tick infestation in livestock and domestic animals is equally important as tick infestation causes significant losses in livestock (cattle, rabbits, guinea pigs) and discomfort to domestic animals. Losses in cattle arise from damage to hides, loss of productivity, anaemia, death and weakness leading to greater mortalities (Merck Veterinary Manual, 1998). The tick *Dermacentor variabilis* which transmits *Rickettsia rickettsia* causes tick paralysis in cattle, dogs, cats as well as humans (Dryden and Payne, 2004). The tick *Ixodes scapularis* has also been implicated in the transmission of *Borrelia* sp. in cats, dogs in addition to the reported cases in humans. *Argas vespertilionis*, is known to transmit *Borrelia* sp., *Rickettsiae* sp. and *Ehrlichia* sp. in bats

(Socolovschi *et al.*, 2012). Hoogstraal (1952) noticed that the nymphs and adult of *A. vespertilionis* bite humans in caves hence they have the potential to transmit pathogens from bat ticks to humans.

5.2 Ectoparasitic prevalence on bat species

Some species of bats harboured a high number of a particular kind of ectoparasite than other species of bats, indicating a high degree of host specialization among these parasites. In terms of bat flies, *R. aegyptiacus, L. angolensis* and *E. helvum* were more heavily infested than the other species of bats and this may be attributed to the roosting habits of the three bat species. *Eidolon helvum*, for instance, is the most widely distributed fruit bat in Africa which tend to live in groups of over 100,000; roosts can build up to millions at the same place at a time (Mickleburgh *et al.*, 2008). This results in individual bats being very close to each other for easy transfer of parasites across members of the colony. This may be the reason for the high number of parasites harboured by *E. helvum*. *Rousettus aegyptiacus* roost in caves (Albayrak *et al.*, 2008) which create favourable conditions for the spread of parasites among these bats since there could be hundreds of bats in a single cave at a time. Despite the few individuals of *L. angolensis* captured, a high proportion of them were infested with bat flies (70.59%). *R. aegyptiacus* and *L. angolensis* appeared to share similar parasites, specifically bat flies. Weber and Fahr (2006) indicated that *L. angolensis* also depends largely or exclusively on the availability of caves as day roosting sites so this would explain why the two species share similar parasites. Until recently, the two species were considered to be one species until they were separated into different species on the basis of the differences in the use of the limbs, and the absence of echolocation in *L.* angolensis (Lawrence and Novick, 1963).

5.3 Ectoparasite infestation prevalence in different sexes and age groups of bats

A number of studies found female bats to harbour more ectoparasites than male bats (Schalk and Forbes, 1997; McCurdy *et al.*, 1998; Morales- Montor *et al*., 2004; Lučan, 2006), but in the current study, male bats were found to harbour more bat flies than female bats. Other studies that have reported higher infestation in male bats than female bats include Moore and Wilson (2002) and Morrand *et al*. (2004). The higher burdens of ectoparasites in female bats has been attributed to immunosuppression during reproduction and aggregation in nursery colonies (Christe *et al*., 2007). However, another theory postulates that abundance of ectoparasite on bats is influenced by host body size, sex and age of bat. Based on this theory that larger habitats support more individuals, and as a result, more species than smaller habitats (Rosenzweig, 1995), Presley and Willig (2008) hypothesize that larger hosts (male bats) would be expected to harbour more parasites than smaller hosts (female bats) since male bats are generally larger than female bats. Flemming (1988) provides a more plausible explanation based on the fact that male bats may have more than one harem in different shelter sites while females are isolated in harems, thus minimizing the exposure of females to bat flies.

With the above inference, it is interesting to note that some studies have reported juvenile bats to harbour more parasites than adult and sub-adult bats (Christe *et al*., 2000; Lučan, 2006). Ectoparasites are transferred to the pups when female bats care for them during the breeding season and as the pups may be inexperienced in grooming, they have minimal ability to reduce ectoparasite burdens. With regards to mite and tick infestations on bats, sex and age of bat were not observed to influence intensity of parasite load.

A number of studies found reproductive females (pregnant and lactating) female bats to harbour more ectoparasites than non-reproductive females (Schalk and Forbes, 1997; McCurdy *et al*., 1998; Morales-Montor *et al*., 2004). During pregnancy and lactation, the immune system of female bats tend to be compromised (Zenon and Hugh, 2011) to allow for the foetus to be successfully carried to term and subsequently breastfed properly. It is possible that parasites take advantage of this compromise in immune competence and parasitize pregnant and lactating females which may not have enough time to groom in order to reduce ectoparasite abundance. As such they are most likely to harbour more ectoparasites than non-reproductive females. There was no such evidence in this study as there was no significant difference in ectoparasite infestation between reproductive and non-reproductive females. Schad *et al.* (2012) and Komeno and Linhares (1999) made similar observations where they found no significant difference in infestation of ectoparasites between reproductive and non-reproductive female bats.

5.4 Ectoparasite infestation prevalence at the different roosting sites

Buoyem had the most diverse ectoparasite occurrence among all the capture sites. The site had a wide variety of vegetation that supported a highly diverse bat population (eight species of bats) and also a wider variety of ectoparasites. Majority of *R. aegyptiacus* bats from which most of the bat flies were collected, were captured from the Buoyem site. The two rare bat flies (*E. africanum* and *E. vandeuseni*) also were collected from *R. aegyptiacus* and *L. angolensis* captured in Buoyem and were absent from Tanoboase Sacred Grove although these two sites are geographically not far apart (N 07º 43.416′, W001º 59.266′ and N 07º 39.942′, W 001º 51.448′ respectively).

The highest number of bats infested with ectoparasites, however, was recorded at the Tanoboase Sacred Grove (62.96%) and the least at the '37' Military Hospital (22.92%). However, a higher proportion of bats captured at Buoyem were infested with bat flies (31.30%), probably because most of the bat species (*R. aegyptiacus* and *L. angolensis*) which harboured bat flies were captured at this site. On the contrary, the highest proportion of bats infested with mites was recorded at the Tanoboase Sacred Grove (33.33%). The Tanoboase Sacred Grove and Buoyem study sites had roosting sites that were mainly caves while the roosting sites at the '37' Military Hospital and Ve-Golokuati comprised of trees. Hence, the higher parasites burdens at the Tanoboase Sacred Grove and Buoyem study sites. This outcome is not surprising as these two capture sites are similar. Bats captured at the Tanoboase Sacred Grove harboured a significantly heavier load of bat flies than bats captured at the other three study sites (X^2= 91.10, df = 3, p-value < 0.01).

5.5 Seasonal patterns of ectoparasite load

Seasonal differences in ectoparasite abundance on bats were observed and were influenced by various factors. Months in which bats were captured were categorized into beginning of rains (March and April) and short dry season (July and August). At the beginning of the rains, bats had higher burdens of ticks as compared to the short dry season probably due to the influence of temperature and humidity. The current results indicated an abundance of ticks that coincided with the onset of the rainy season and declined steadily as the season progressed to the end of the rainy season. Climate is known to be a better predictor of tick distribution than other factors such as vegetation type (Cumming, 2002). In the temperate regions, parasites are usually abundant in the summer, when the temperature is warm than in the winter when it is cold (Lučan,

2006) and tick development has been reported to be influenced by humidity and temperature variations (Ntiamoa-Baidu, 1987a; b; Olenev, 1927).

Most of the ticks recorded in this study were collected in the month of April and were mostly *Argas vespertilionis*. Hosseini-Chegeni and Tavakoli (2013) established the optimum temperature for growth of *A. vespertilionis* to be between 22 – 28ºC at 80% relative humidity. In Ghana, the average temperature (24 - 28ºC) and relative humidity (80%) in April fall within the optimal temperature and relative humidity for *A. vespertilionis* development. Milne (1948) and MacLeod (1934) also noted that unfed ticks require a relative humidity above 80% to survive, with anything less having a detrimental effect on their survival.

Mites are very diverse with so many species that may require a range of temperature and humidity conditions for optimal reproduction and survival. Mite distribution show clear seasonal patterns in the temperate regions but not in the tropics. Although it is cooler in the rainy season than it is in the dry season in the tropics, the temperature difference is not as steep a change as compared to the sharp temperature difference experienced in the temperate regions (Houghton and Woodwell, 1989). This may account for the reason why mite abundance and intensity was not affected by seasonal changes.

The highest prevalence of bat flies was recorded in the month of March, which marks the beginning of the rainy season in southern Ghana. From all indication, bat fly infestation was at its peak before the rains began. It is possible that the rains may have washed away some of the bat flies harboured by bats as Pilosof *et al*. (2012) indicated that precipitation may directly affect the survival of fly pupae hence the low numbers of bat flies during the rains.

Another interesting observation was made of bat flies which were not recorded in Ve-Golokuati until in August. Seasonal flowering of plants (*Ficus elastica*) and fruits (guava, mango) on which bats feed on may account for this. Some fruites on which bats feed on flower and fruit seasonally and will therefore not be available to bats throughout the year. An example of such fruits include *Ficus elastica,* an important food source for bats which flower from March to April (Mahbubur and Khanom, 2013) and the fruits will therefore be depleted by August. Bats therefore move further away from their roosts in search of food and by so doing disperse parasites to other roosting sites. It is assumed that the few individuals of the bat species *R. aegyptiacus* and *E. helvum,* on which the few bat flies were collected from, may have come form roosts further away from Ve-Golokuati.

5.6 Endoparasite distribution

5.6.1 Endoparasites prevalence of bat species

Bats are not only infested with ectoparasites but endoparasites as well. Bats had a surprisingly high infection with haemoparasites corresponding to an overall prevalence of 47.71% (128 out of 262). This result is similar to a study done by Schaer *et al*. (2013), which also reported approximately 40% prevalence of bats infection with haemoparasites. Kudo (1966) suggested that since arthropods, specifically bat flies, are known to transmit heamoparasites especially bat malaria, it would be expected that individuals heavily infested with bat flies would be highly infected also with haemoparasites. This was confirmed as *R. aegyptiacus, L. angolensis* and *E. helvum* which had heavy infestations with bat flies were also highly infected with haemoparasites.

On the contrary, *E. gambianus,* which had a low bat fly infestation, was highly infected with

haemoparasites than *E. helvum* which were heavily infested with bat flies. This suggests haemoparasite transmission from sources other than the bites of bat flies. Haemoparasite infection could be influenced by the eating habits of the bats (Kudo, 1966) where fruit-eating bats sometimes supplement their diet with insects which provide them with the nutrients, particularly proteins that they do not get from feeding exclusively on fruits (Thomas, 1984; Herrera *et al.,* 2001). Bats captured at the '37' Military Hospital, although not heavily infested with bats flies, carried the heaviest infection with haemoparasites.

Infection with haemoparasites seemed not to be affected by sex, age, species or roosting site of bats but was influenced by reproductive status of female bats. Hence, although there was no significant difference in ectoparasite infestation between reproductive and non-reproductive females, lactating female bats tended to have heavier infection with haemoparasites than pregnant and non-reproductive females. This may be, as noted earlier, due to the immuno-incompetence of female bats during pregnancy and lactation (Zenon and Hugh, 2011).

Several studies (eg. Kamani *et al*., 2014) have reported *Bartonella* sp., a bacteria infection, and bat flies on the same bat. The bat flies get infected as they continuously feed on the bacteria infected bats and may transmit the pathogens through urine, droppings and saliva to humans when they bite humans (Wenzel *et al*., 1966). Nycteribiid bat flies also transmit *Polychromophilus* spp. to bats (Gardner and Molyneux, 1988) therefore are of more concern compared to the streblid bats flies which have not been implicated in the transmission of haemoparasites. In the case of this study, all bat flies recorded were of the family Nycteribiidae hence a high possibility of pathogen transmission to humans.

Transmission of trypanosomes is likely also to occur by an oral route when the vector insects are eaten by insectivorous bats (Lima *et al*., 2012). Insectivorous bats would therefore be more

likely carriers of trypanosomes than fruit-eating bats. Bat bugs of the family Cimicidae have been known to harbour and transmit trypanosomes among bats and the grooming habits of the bats probably facilitate transmission (Marinkelle, 1976; Gardner and Molyneux, 1988; Molyneux, 1991). The absence of trypanosomes in bats in this study would be because of the absence of bat bugs except for a single specimen collected from an *Epomophorus gambianus* in Buoyem. According to Cavazzana *et al*. (2010), Hoare (1972), Marinkelle (1976) and Molyneux (1991), there is no evidence that trypanosomes cause harm to bats. However, it is very possible that these bats can be reservoirs for pathogenic trypanosome species which could harm humans.

5.6.2 Molecular analysis

Samples that tested positive for haemoparasites by microscopy were molecularly tested to determine the species of *Plasmodium* in order to ascertain the impact of haemosporidian parasite on bat biodiversity and possibly the role of bats as hosts of zoonotic malaria. The haemoparasite of interest was the *Plasmodium* species which is the species of protozoan that causes malaria in people. The agarose gel electrophoresis done yielded no results and further studies could not be done. DNA extraction was done using the Chelex protocol of Wooden *et al.* (1993) which was a reliable method of extraction. The lack of results may be because the haemoparasites observed were not of the genus *Plasmodium* sp. but rather of other genera (eg. *Nycteria* sp. or *Hepatocystis* sp.) that also infect bats. Several tests were run on samples to confirm the results. A lot of troubleshooting was done to determine the shortcomings but none yielded results. The microscopy showed that parasitemia was generally low with just a few cells showing high was infection. The DNA extracted would therefore be very little which could be the reason why it was not detected when an agarose gel electrophoresis was run on the samples.

The volume of DNA was exponentially increased in each PCR cycle to cater for these low levels of DNA. In the study done by Schaer *et al*. (2013), PCR was done using illustra* PuRe Taq Ready-To-Go PCR beads (GE Health Sciences), which was reported to be more sensitive and could determine very low concentrations of DNA. This however was not readily available for the study and therefore the conventional form of PCR was used. Perhaps PCR done using the normal PCR machine could not detect and amplify the low concentration of DNA enough for detection. The cycling conditions for PCR were also changed in each cycle but this also did not yield any results. Initially 35 cycles was used followed by 40 cycles. Throughout all these changes, the control which had thousands of malaria parasites was visible when an agarose gel electrophoresis was run. It was concluded that either the DNA extracted was too little to be detected or the DNA extracted was not that of *Plasmodium* sp..

Apart from haemoparasites that were of interest, some bacteria were also observed in the blood. Although the study of bacteria was not an aim of the study, it would be interesting to study the species of bacteria that is harboured by bats since humans can also be plagued with bacteria harboured by bats. Bats are known to harbour bacteria such as *Bartonella* sp. (Loftis *et. al*., 2005), *Rickettsia* sp. (Diaz, 2010), and *Borrelia* sp. (Sonenshine, 1991) which can infect humans.

5.6.3 Helminth Infestation

Bats are known to be infected with helminthes but in this study, none of the faecal samples of the twenty nine bats screened molecularly showed any helminth infestation. Several studies of helminth infections of bats have focused on insectivorous bats (Esteban *et al*., 1999; Okafor *et al*., 2004; Tinnin *et al*., 2008; Junker *et al*., 2008) and a few on fruit-eating bats (Nogueira *et*

al., 2004) where most of the helminthes were sampled from the gastrointestinal tract. This difference in method could be a reason why helminthes were not detected in the bat stool samples examined in this study. Due to the fact that this study was under a bigger project, Dynamic Drivers of Disease in Africa Conservation (DDDAC), whose main aim was to conserve bats, it was not possible to kill bats in order to observe gut contents. Therefore a less intrusive method was used which was to sample faecal pellets and observe if there were any helminth eggs in them.

The prevalence and incidence of helminthes are strongly influenced by the feeding habits and foraging strategies of bats (Marshall and Miller, 1979), with most of them found in insect-eating bats, that are prone to ingest an infected insect serving as intermediate host, than in fruit-eating bats (Coggins, 1988). In a study by Saoud and Ramadan (1976), none of the *R. aegyptiacus* bats captured harboured any helminth parasite, probably because these were strictly fruit eating bats. This could be the reason why none of the screened bats assessed in this study had any helminth infections since all were fruit eating bats. It could also be explained in that these bats may have had either a single male or female adult infection and therefore there could not be any reproduction to produce eggs. It could also be that bats harboured adult helminths that may have exceeded their reproductive age hence the parasites shedding no eggs to be detected by PCR.

Not only do the intestines of bat sustain various helminths but diverse microbiota as well, including insects, mites, bacteria and fungi (Estrada-Bárcenas, 2010). Hence further studies are required to shed light on the role these groups of pathogens play in bat biodiversity and the potential transmission of zoonotic diseases to humans.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATONS

6.1 Conclusions

The findings of the study indicated that bats indeed harbour a wide array of ectoparasites (bat flies, mites and ticks) and endoparasites. Four species of bat flies, eighteen species of mites, two species of ticks and a bat bug were collected from the 480 bats examined. Two unidentified mites were also recorded. Mites were the most abundant ectoparasite recorded on all species of bats. From literature, some of these ectoparasites were vectors of diseases of public health importance to humans. For example bat flies have been implicated in the transmission of malaria and mites in the transmission of bacteria which cause allergies in humans among others.

Ectoparasites were collected from all four study sites. Buoyem, however had the most species of ectoparasites and the '37' Military Hospital the least. Adult bats were significantly infested with more bat flies than sub-adult and juvenile bats. There was no significant difference in mite and tick infestations in the different age categories of bats. Male bats were significantly infested with bat flies than female bats. There wass no significant difference in infestation with ticks and mites between male and female bats. There was a significant difference in ectoparasite infestation between reproductive and non-reproductive female bats.

Bats captured at the Tanoboase Sacred Grove harboured the highest load of bat flies and mites while bats captured at the Ve-Golokuati harboured the highest load of ticks. A relationship between month of capture and ectoparasite infestation was established where bats captured in March had the heaviest bat fly load. The heaviest mite and tick loads were recorded in the months of July and April.

Approximately 48% of the total number of bats, comprising of all nine species, were infected with haemoparasites. A higher proportion of male bats were infected with haemoparasites than female bats. There was no significant difference in infection among the different age categories. Also, bats captured at the '37' Military Hospital were heavily infected than bats captured at the other sites. The highest proportion of bats infected with haemoparasites was captured in August while the least proportion was recorded in July. Here, lactating female bats were significantly infected with haemoparasites than pregnant and non-reproductive females. Finally, none of the bats captured were infested with helminths based on examination of faecal samples.

In summary, bats were noted to harbour parasites (bat flies, mites, ticks, bat bug and haemoparasites) of noticeable public health importance.

6.2 Recommendations

- To the best of my knowledge, for the first time, several ectoparasites comprising of four bat fly species, twenty mite species and two tick speces have been isolated from bats in Ghana. These ectoparasites may be of public health significance and also of importance in relation to bat conservation efforts. Further studies are thus required to shed light on the importance of these parasitic infestations to public health and biodiversity conservation efforts. Additionally, more extensive studies at other locations in Ghana are required to give a more complete overview of bat ectoparasites towards creating a national database.
- The major haemoparasite observed was bat malaria parasites; a few bacteria infections were also observed. Due to logistical and time cosntraints, an extensive study could not be carried out to investigate further the specific species of malaria parasites. I would

therefore recommend studies be carried out in order to identify the exact species of haemoparasites infecting bats in Ghana.

- Although bats are known to harbour parasites of zoonotic importance which could potentially be vectored by ectoparasites, very little or no information exists on the role of these arthropod ectoparasites (including those found in the present sudy) in pathogen transmission either from bat to bat or bat to human. As such additional investigations are required to determine the vectoral role of bat ectoparasites in disease transmission to humans and animals, if any.

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