

# A SURVEY ON ENDOPARASITES OF DOGS IN LUSAKA AND KATETE DISTRICTS OF ZAMBIA.

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By

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A thesis submitted to the University of Zambia in fulfillment of the requirements for  
the degree of Master of Science in Infectious Diseases

**The University of Zambia**

**Lusaka**

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**DECLARATION**

I, **Eugene C. Bwalya** do hereby declare that the contents of the thesis being submitted herein are my original work and they have not been previously submitted to any university for the award of a degree or any other qualification.

Signature.....

Date.....

**CERTIFICATE OF APPROVAL**

This thesis submitted by **DR. Eugene Chisela Bwalya** is approved as fulfilling the requirements for the award of the degree of Masters of Science in infectious diseases by the University of Zambia.

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## ABSTRACT

The present study attempted to bridge the paucity of information on the prevalence of canine endoparasites in Zambia and to establish some of the risk factors that may be associated with endoparasitism. Faecal, blood and necropsy data on endoparasites were collected between January and December 2010 to determine the prevalence of canine endoparasites in two demographically and socio-economically diverse regions of Zambia. A total of 486 dogs were sampled ( $n = 160$  Katete, predominantly rural;  $n = 326$  Lusaka, predominantly urban). Faecal samples were examined by simple and centrifugal faecal flotation methods for the presence of helminth eggs. In Katete, 82.5 percent of dogs were positive for gastrointestinal (GI) helminths compared to 76 percent for Lusaka. The overall prevalence of single helminth infections were as follows: 72.1 percent *Ancylostoma caninum*, 11.3 percent *Toxocara canis*, 6.6 percent *Trichuris vulpis*, 6.2 percent *Dipylidium caninum*, 4.4 percent *Toxascaris leonina* and 0.7 percent Taeniid eggs.

Except for *T. vulpis* (Lusaka: 0.3 percent, Katete 18.1 percent) and *D. caninum* (Lusaka: 3.8 percent, Katete: 13.1 percent) ( $p < 0.05$ ), the results indicated no significant difference in the prevalence of the detected helminths between Lusaka and Katete. In this study, there was no significant difference in GI helminth prevalence between sexes except with *A. caninum*, which showed a significantly higher prevalence of 80.5 percent in dogs aged between six to 12 months old in comparison to the 56.1 percent in those above 60 months of age. The prevalence of multiple infections with two and three parasites species per host was not statistically different between Lusaka and Katete except for *A. caninum* single infections and co-infection with *D. caninum* ( $p < 0.05$ ).

Thirty-three dogs destined for euthanasia were collected and necropsy information collected. *A. caninum* was the most prevalent (93.9 percent) helminth recovered in euthanized dogs with *T. canis* being the least (6.1 percent). The prevalence of *D. caninum* was 63.6 percent whereas that of *Spirocerca lupi* was 27.3 percent.

Thin blood smears stained with Giemsa and buffy coats were examined for parasites. The prevalence of *Babesia canis* was very low and was only reported in Lusaka. There were no positive cases of canine trypanosomosis.

Significant reduction in packed cell volume (PCV) was observed with two co-infecting GI helminths in Katete and overall, eosinophilia was observed only for *T. leonina*.

The presence of the zoonotic helminths *A. caninum*, *T. canis* and *D. caninum* in these two demographically and socio-economically diverse regions of Zambia indicates that dogs play an important epidemiological role as reservoirs of infections for man.

## **DEDICATION**

*This work is dedicated to my parents, Emmanuel and Jennifer Bwalya, my wife Dr. Chanda Chitala-Bwalya and our daughter, Jennifer Maambo Bwalya.*

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## **LIST OF ABBREVIATIONS**

<b>ANOVA</b>	Analysis of Variance
<b>CF</b>	centrifugal flotation
<b>CI</b>	Confidence Interval
<b>DG</b>	dark ground
<b>dl</b>	decilitre
<b>DVO</b>	District Veterinary Officer
<b>EDTA</b>	Ethylene Diamine Tetraacetic Acid
<b>ELISA</b>	Enzyme Linked Immunosorbent Assay
<b>GI</b>	gastrointestinal
<b>HCT</b>	haematocrit centrifuge technique
<b>HIV/AIDS</b>	Human Immunodeficiency Virus/Acquired ImmunoDeficiency Syndrome
<b>LAMP</b>	Loop Mediated Isothermal Amplification
<b>LAWS</b>	Lusaka Animal Welfare Society
<b>n</b>	Sample size
<b>PA</b>	parasitic agent
<b>PACRA</b>	Patent Companies Registration Agency
<b>PCR</b>	polymerase chain reaction
<b>PCV</b>	Packed Cell Volume
<b>RPM</b>	Revolution per minute
<b>SF</b>	Simple flotation
<b>sp.gr.</b>	Specific gravity

<b>SPSS</b>	Statistical package for social scientists
<b>TPP</b>	Total plasma protein
<b>UNZA</b>	University of Zambia
<b>Unzavet</b>	University of Zambia veterinary clinic

## CHAPTER 1 INTRODUCTION

Parasitism exerts deleterious effects and poses serious health problems in domestic dogs (*Canis lupus familiaris*), such as retarded growth, generalized ill health, lowered resistance to infectious disease and reduced work efficiency (Qadir *et al.*, 2010). Like other mammalian hosts, dogs are susceptible to infection with a range of intestinal parasitic helminths and protozoa, including species of epidemiological significance that can also be a source of severe disease for humans (Bajer *et al.*, 2010). Similarly, dogs can be infected with pathogenic haemoparasites like certain *Babesia* species (Nalubamba *et al.*, 2011) and *Trypanosoma* species (Keck *et al.*, 2009).

Very few dogs in Zambia are routinely administered anthelmintic drugs, but are often allowed to roam the streets and live in close proximity to humans. This means that the local population is exposed to a broad spectrum of zoonotic parasites by means of environmental contamination with dog faeces.

Dogs are the most successful canids adapted to human habitation worldwide. They have contributed to physical, social and emotional well-being of their owners, particularly children (Dohoo *et al.*, 1998; Robertson *et al.*, 2000). However, despite the beneficial effects, close bonds of dogs and humans remain a major threat to public health, with dogs harbouring a bewildering number of infective stages of parasites transmissible to man and other domestic animals (McCarthy and Moore, 2000; Molyneux, 2004; Robertson *et al.*, 2000). For example, well-known and important zoonotic parasitic diseases are cutaneous and visceral larva migrans, hydatid disease and tungiasis (Akao and Ohta, 2007; Heukelbach *et al.*, 2003). In low-income settings, treatments to eliminate these parasites are (if done at all) often applied in advanced

stages of disease, causing distress to pets and their owners (Irwin, 2002; Morrison, 2001).

Throughout their long history of domestication, dogs have been sources of zoonotic parasites and have served as a link for parasite exchange among livestock, wildlife, and humans (Macpherson, 2005). Globally, dogs remain an important source of emerging diseases in humans such as eosinophilic enteritis caused by *Ancylostoma caninum*, a bridge for reemerging infections such as *Echinococcus multilocularis* and a source of opportunistic parasites such as *cryptosporidium* for immunocompromised persons (Macpherson, 2005). The close relationship between dogs and humans, the numerous uses to man of these companion animals and their ubiquitous distribution has resulted in them unwittingly participating in sharing over 60 parasite species including *Giardia*, *Cryptosporidium*, *Toxoplasma*, *Echinococcus* spp., *Ancylostoma* and *Toxocara* (Eguia-Aguilar *et al.*, 2005). Clearly, interaction between humans and dogs is an important factor in the epidemiology of zoonotic diseases harboured by dogs. It is a fact that human-dog companionship is a natural relationship and thus controlling parasitic zoonotic diseases communicable between man and dog is a big challenge. One of the strategies of controlling parasitic zoonotic diseases would be through changing human behavior as it relates to companionship with dogs and encouraging the taking of precautionary steps such as proper sanitizing/washing of hands before eating food. However, changing human behaviour such as improving hygiene levels, providing of safe drinking water and the proper use of sanitary facilities will remain as challenging as controlling stray and feral pet populations (Macpherson, 2005).

In many African countries, including Zambia, appropriate policies regarding pet ownership and their effects on individual and community health are nonexistent. Prevalence of parasite infection in dogs with importance for human health is usually high, resulting in risk of zoonotic transmission from dogs to humans (Ugbomoiko *et al.*, 2008). The risk is further increased by non-favourable ecological and human behavioural factors such as consumption of undercooked pork meat (Malgor *et al.*, 1996).

Today, in many African countries, veterinary services are absent or restricted, and disease surveillance programs and routine preventive health measures such as vaccination and parasite control are rare (Ugbomoiko *et al.*, 2008). These conditions have limited our understanding of disease interactions at the dog-human interface and our ability to detect and respond to emerging diseases.

Few studies have been conducted on intestinal parasitism in dogs in Zambia. The first study was by Islam and Chizyuka (1983) who investigated the prevalence of helminth parasites of dogs and recently Nalubamba *et al.* (2011) investigated the epidemiology of canine *Babesia* infections. Both investigations were conducted in Lusaka District. Thus, a lot of information on canine parasitism is generally lacking in Zambia despite most veterinary clinics and research centres conducting routine identification of these parasites. The aim of the present study was to determine the prevalence of endoparasites in two demographically and socio-economically diverse regions of Zambia in order to improve our understanding of the epidemiology of zoonotic endoparasites of canine origin.

## **1.1 Study rationale**

Understanding the epidemiology of canine endoparasites is important in the formulation of canine endoparasite control programs. However, in Zambia, very little is known about the epidemiology of endoparasites in the dog population, making control extremely difficult. This situation is further compounded by the current scenario in which over the past few decades there has been an increase in the dog population, which has inevitably resulted in an increase in dog-human interactions. With the HIV-AIDS pandemic, which has undoubtedly increased the susceptibility of the human population even to less virulent parasites harboured by dogs, this paucity of epidemiological information on canine endoparasites has serious implications for the risk of zoonotic parasite transmission from dogs to humans. Thus, this study investigated the prevalence of canine endoparasitic diseases with a view to better understand the epidemiology of these diseases.

The epidemiology of endoparasitic diseases of dogs is normally influenced by demographic and socio-economic factors. It is thus important when investigating the epidemiology of such diseases to consider dogs demographic and socio-economic factors. The socio-economic factors include things such as unequal distribution of veterinary services between urban and rural areas in addition to the living conditions that are particularly poor in the rural communities compared to urban communities. According to the June, 2009 Patent Companies Registration Agency (PACRA) register of business companies, Lusaka urban had 30 registered companies that were providing veterinary services to the communities. This is contrary to the situation in Katete district that is predominantly rural with mainly agricultural-based economy, which has

one government district veterinary office. It is therefore generally expected that a number of people who own dogs in Lusaka urban to have more access to veterinary services for their pets. On the other hand, the number of veterinary clinics limits access to veterinary services for people in rural Katete. Therefore, it is likely that most dogs in these rural areas have never been treated for any form of parasitic diseases.

Hitherto, no study on zoonotic canine endoparasitic diseases was done in Zambia that took into account demographic and socio-economic factors. Findings in this study will undoubtedly contribute to the formulation of rational control measures for canine endoparasitic diseases.

## **1.2 Objectives**

### **1.2.1 General objective**

To investigate the prevalence of endoparasites in dogs in Zambia and the potential for zoonosis.

### **1.2.2 Specific objectives**

- To determine the spectrum and prevalence of endoparasites in dogs in Lusaka district, a predominantly urban setting, and Katete district, a predominantly rural setting.
- To determine the prevalence of canine zoonotic endoparasites in Lusaka and Katete districts.
- To compare the prevalence and spectrum of canine zoonotic endoparasites in Lusaka and Katete districts.
- To determine the effect of endoparasitism on packed cell volume (PCV), eosinophil count and total plasma protein (TPP).

### **1.3 Null hypothesis**

1. Dogs in urban Lusaka and rural Katete harbour endoparasites of which some are potential agents of zoonosis.
2. There is no significant difference in the prevalence of canine endoparasites between the urban Lusaka and rural Katete.

### **1.4 Alternative hypothesis**

1. Dogs in urban Lusaka and rural Katete do not harbour endoparasites of potential zoonosis

## **CHAPTER 2 LITERATURE REVIEW**

### **2.1 Dog domestication and relationship to Man**

The importance of the high prevalence of intestinal parasites in dogs, the close contact of humans with dogs' excreta and the possible role of this environmental pollution in the spread of human disease has been described previously (Seah *et al.*, 1975). The dog was the first animal to be domesticated and the process started at least 15 000 years ago (Bjornerfeldt, 2007). Today it is the most morphologically diverse mammal, with a huge variation in size and shape. Dogs have always been useful to humans in several ways, from being a food source, hunting companion, guard, social companion and lately also a model for scientific research (Bjornerfeldt, 2007). Dog history is really the partnership between dogs and humans. Dogs get companionship, protection and shelter, and a reliable food source out of the deal. As to when this partnership first occurred is now controversial. Current research indicates that domestication, or the attributes of a domesticated animal, can occur much more quickly than previously believed, even within a human generation or two with determined selective breeding. It is also now generally believed that initial domestication was not attained deliberately by human intervention but through natural selection. Wild canines who scavenged around human habitation received more food than their more skittish counterparts. Those who attacked people or their children were probably killed or driven away, while those more tolerant animals survived.

Man has been fond of the dog throughout the ages. Dogs are depicted on early rock drawings and on friezes and pottery. They are carved on tombs, particularly those of the Crusade knights, and on monuments such as the Scott Memorial and "Greyfriars

Bobby" in Edinburgh, United Kingdom (Bennet, 1977). Dogs are found almost everywhere in modern-day society. For some people, dogs are an essential part of their public image. In many societies, a dog-beater is likely to be much more severely punished than a child-beater. Men are generally observed to be more careful of the breed of their horses and dogs than of their children (Bennet, 1977).

## **2.2 Dog population in the world**

Accurate figures of pet populations are difficult to obtain, but enough statistics are available to provide a reliable estimate. In the United States of America (USA), the dog population was estimated around 40 million while that of Canada was about 4 million (Bennet, 1977). Furthermore, 5000 dogs are born every hour in the USA alone (human births, 200 to 300 per hour) (Beck, 1975). In 1973, dog food worth over \$2 billion was sold in the USA. It was said that in Canada, Toronto had two million people and one million dogs and cats; Montreal had a similar ratio (Bennet, 1977). More recent pet population estimates of top 10 countries include the USA with 61,080,000, Brazil 30,051,000, China 22,908,000, Japan 9,650,000, Russia 9,600,000, South Africa 9,100,000, France 8,150,000, Italy 7,600,000, Poland 7,520,000 and Thailand with 6,900,000, ([www.mapsofworld.com](http://www.mapsofworld.com)), Accessed on 03/04/2010).

The dog population in Zambia is unknown, although some districts and towns have some estimates but this information is generally lacking and remains a concern of worry with the ever-increasing stray dog population and unplanned human settlements. A 2006 estimate from a dog census in Katete District, one of the rural towns in Zambia, indicated a population of 10,000 dogs (Personal communication, Dr. E. Chanda, District Veterinary Officer [DVO], Katete). However, of the 10,000-dog

population reported, the district only vaccinates an average of 4,000 dogs against rabies every year. The District Veterinary Office, Lusaka, has no records concerning the dog population. This is mainly due to the absence of a Ministry of Local government dog register census and failure of most registered veterinary clinics to submit their annual rabies vaccination books from where the office makes its estimates. However, according to their compilation for the number of dogs that were vaccinated against rabies for 2009, the office recorded 2,069 (Personal communication, Dr. M. C. Kanemanema, [DVO], Lusaka). This however is not indicative of the total dog population in Lusaka urban as other dogs are vaccinated from various veterinary clinics within Lusaka. The 2,069 includes dogs that were vaccinated in the high density, low-income residential areas by some non-governmental organizations and private veterinary clinics. The University of Zambia veterinary clinic recorded 2,721 dogs that were vaccinated against Rabies for the year 2009 (unpublished official records).

### **2.3 Canine endoparasites**

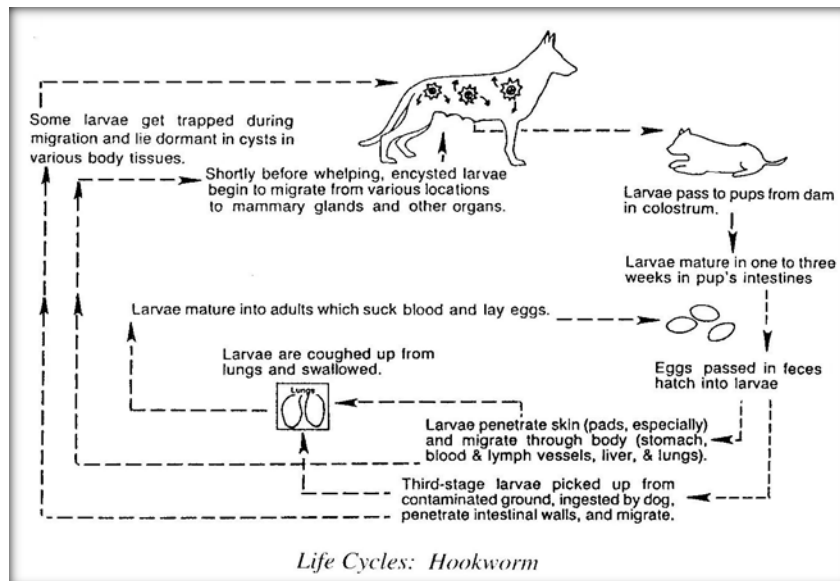
Canine parasitoses may be caused by ectoparasites such as ticks, fleas, lice, and mites or endoparasites which include protozoan parasites like *Trypanosoma* and *Babesia* species and helminths like *Toxocara*, *Echinococcus*, *Trichinella* and *Ancylostoma*). Most of these endoparasites are potential agents of zoonoses (Ugbomoiko *et al.*, 2008). Endoparasites of dogs can be broadly divided into gastrointestinal (GI) parasites and haemoparasites.

#### **2.3.1 Gastrointestinal parasites**

This group comprises GI helminths and protozoan parasites.

### 2.3.1.1 Gastrointestinal helminths

GI helminths belong to two major phylums namely, Nematelminthes (roundworms) and Platyhelminthes (flatworms) (Kassai, 1999). Though the phylum Nematelminthes has six classes, only one of these, the nematoda, contains worms of parasitic significance. In the nematoda, the sexes are separate and the males are generally smaller than the females, which lay eggs or larvae (Soulsby, 1982). One feature of the nematode life cycle is that immediate transfer of infection from one final host to another rarely occurs. Some development usually takes place either in the faecal part or in a different species of animal, the intermediate host, before infection can take place (Urquhart *et al.*, 1987). An example of such a life cycle, exemplified by the hookworm is illustrated in Figure 2.1.

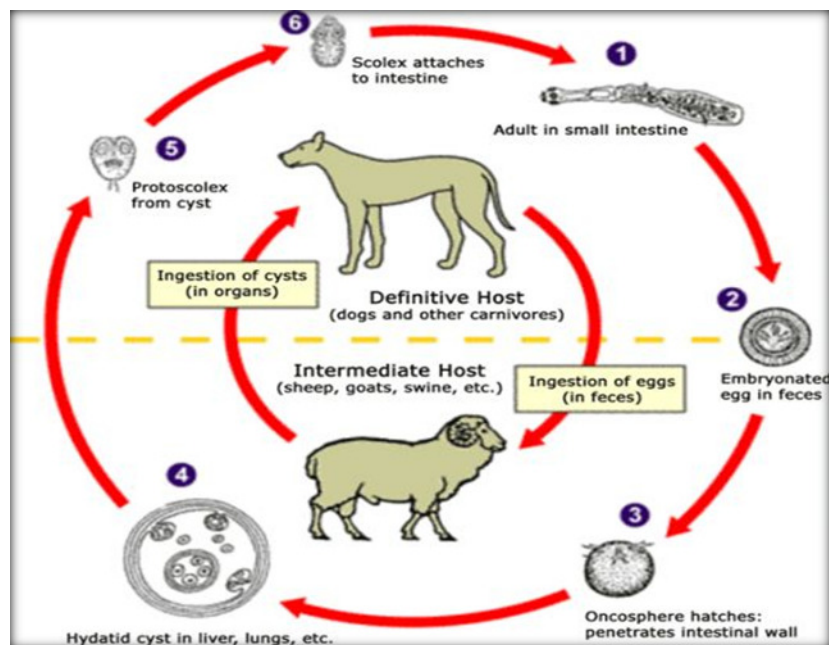


(Sourced from: <http://www.thedogplace.org/DogCare/0811-DogWorms-hookworms> 03/02/2011)

Figure 2.1: life cycle of hookworm in dogs

Platyhelminthes contains two classes of parasitic flatworms, the Trematoda and the Cestoda. The class Trematoda comprises two main subclasses: the Monogenea

that have a direct life cycle and the Digenea, which require an intermediate host (Urquhart *et al.*, 1987). The former are found mainly as external parasites of fish, while the latter are found exclusively in vertebrates and are of considerable veterinary importance. Almost all the tapeworms of veterinary importance in the Cestoda class are in the order Cyclophyllidea, the two exceptions being in the order Pseudophyllidea (Kassai, 1999). Tapeworm life cycles tend to follow a set pattern, which depends on the presence of a predator-prey relationship (Figure 2.2). The prey differs depending on the intermediate host i.e. fleas (*Ctenocephalidae canis*) are the intermediate host for *Dipylidium caninum*. Eggs laid by the adult pass out in the faeces of the dog and are eaten by the intermediate host. These develop into the intermediate stage or cyst, which generates the adult when eaten by the dog. A tapeworm may pass through several intermediate hosts before it finds the right definitive host.



(Sourced from: <http://niyah.net/gallery2/view.php> 03/02/2011)

Figure 2.2: Life cycle of tapeworms in dogs

#### 2.3.1.1.1 Nematodes

These belong to the class Nematoda. They are free living or parasitic, unsegmented worms usually cylindrical and elongated in shape (Kassai, 1999). Nematodes have an alimentary canal and are commonly called round worms because of their appearance in cross-section (Urquhart *et al.*, 1987). Some of the nematodes that are known to be zoonotic include *Ancylostoma caninum*, *Trichinella spiralis*, *Capillaria aerophila* and *Toxocara canis* (Dai *et al.*, 2009). Parasitized dogs show a variety of clinical signs ranging from asymptomatic dogs to those with clinical signs, depending on the parasite species and density. These clinical signs vary from unthriftiness, malaise, irritability, capricious appetite and shaggy coat to colic, mild diarrhea, melena, vomiting, anorexia, low weight, anemia, dull coat and dehydration with severe infestations being fatal (Lorenzini *et al.*, 2007). *Ancylostoma caninum* is known to cause cutaneous larval migrans in humans. The disease in humans is known to be distributed worldwide in tropics and subtropics and is contracted through contact with infective larvae that penetrate skin. Dogs, cats and wild carnivores are known to be the principal animals involved in the maintenance of ancylostomiasis (Susan and Aiello, 1998). On the other hand, human toxocariasis is caused by roundworms *Toxocara canis* and *T. cati* commonly found in the intestine of dogs and cats respectively. Although infection with these parasites have been described in their usual hosts for more than two centuries, only in the 1950s were they recognized as important human pathogens (Beaver *et al.*, 1952). When embryonated *Toxocara* eggs are accidentally ingested by humans, second-stage larvae (L2) hatch in the small intestine and wander through the body, but fail to develop to mature adult worms

within this aberrant host. The clinical spectrum of human toxocariasis, which varies from asymptomatic infections to severe organ injury, is determined by the parasite load, the sites of larval migration and the host's inflammatory response (Pawlowski, 2001). Nematode worms of the genus *Trichinella* are one of the most widespread zoonotic pathogens in the world. Infection by *Trichinella* spp. has been detected in domestic and/or wild animals of all continents, with the exception of Antarctica, where there is no record of the parasite (Pozio, 2007). Human trichinellosis may be acquired through the consumption of undercooked meat of different animal origin such as pork, horse, dog and game meat containing infective larvae of *Trichinella* spp (Slifko *et al.*, 2000; Pozio, 2007). The global prevalence is difficult to evaluate, but as many as 11 million people may be infected worldwide (Dupouy-Camet, 2000; Slifko *et al.*, 2000). Spirocercosis is a disease occurring predominantly in Canidae and is due to *Spirocerca lupi*, which is worldwide and endemic in some warm climates. Despite the importance of *S. lupi* in causing dysphagia, vomiting, esophageal neoplasia, aortic aneurysm or rupture, and secondary pulmonary osteoarthropathy, there are very few reports on the epidemiology and pathologic lesions of this infection. The important factors affecting prevalence of this disease are proximity to intermediate (dung beetles) and paratenic hosts and the population density of intermediate and infected adult hosts (Oryan *et al.*, 2008). Infection with *Eucoleus (Capillaria) aerophila* also is believed to be via direct ingestion of the egg with an infective larva (Campbell and Little, 1991). The prepatent period is between three to five weeks. The main clinical signs in the dog are coughing and wheezing due to bronchiole disease.

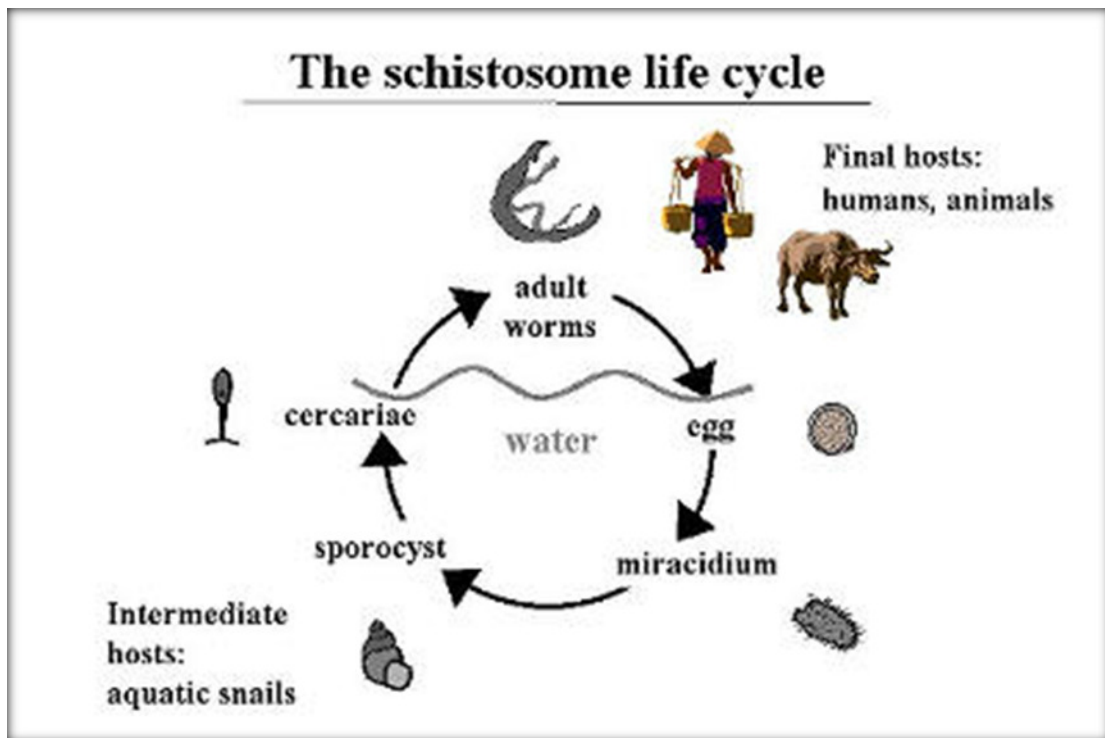
#### 2.3.1.1.2 Cestodes

These are hermaphrodite endoparasitic worms with an elongated flat body and without body cavity or alimentary canal. They may be a few millimeters to several metres in length (Kassai, 1999). There are several zoonotic cestodes including *Dipylidium caninum*, *Echinococcus granulosus*, *Echinococcus vogeli*, *Diphyllobothrum latum*, *Taenia multiceps*, and *Spirometra* species (Susan and Aiello, 1998). Most urban dogs and cats eat prepared foods and have no access to natural prey, but such animals may acquire *D. caninum* from fleas. Suburban, rural and hunting dogs have more access to various small mammals, in addition to raw meat and offals from domestic and wild ungulates. A number of cestodes can be expected in such dogs. On sheep ranges and wherever wild ungulates and wild canids are common, dogs may acquire *Echinococcus granulosus* (the hydatid tapeworm) (Jenkins, 2001). Whereas ingestion of eggs passed in infected dogs' faeces may result in human infection with metacestodes of *E. granulosus*, *Echinococcus multilocularis*, *Taenia multiceps* and *Taenia serialis* in various tissues, accidental ingestion of the intermediate host that contains the cysticercoids (infected fleas) may result in human infection with adult *D. caninum* in the intestine (Elliot *et al.*, 1985; Warner, 1984). Although the risk of human infection is low and often asymptomatic, it is most likely to affect small children because of frequent contact with canine pets in public parks and open areas (Canto *et al.*, 2010).

#### 2.3.1.1.3 Trematodes

These are elongate, unisexual and dimorphic which inhabit the blood vessels of their hosts. They are flattened dorso-ventrally and are commonly called 'flukes'

(Kassai, 1999). *Schistosoma mansoni* occurs in the mesenteric veins of man in Africa, South America and the Middle East and humans are the most important definitive hosts. However, dogs have been found to be naturally infected with *S. mansoni* in East Africa (Soulsby, 1982) whereas *Clonorchis sinensis*, *Echinostoma* species, *Fasciolopsis buski*, and heterophids have been reported mainly in Asia (Susan and Aiello, 1998). The life cycle of *S. mansoni* in dogs follows a similar path as in humans (Figure 2.3) with the intermediate host being *Biomphalaria glabrata*, a member of the ram's horn snail family (Planorbidae, Basommatophora).



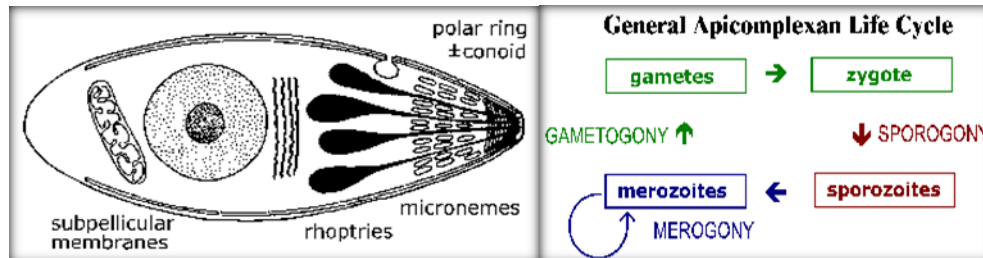
(Sourced from: [http://www.qimr.edu.au/content/image/lifecycle\(1\)](http://www.qimr.edu.au/content/image/lifecycle(1)) 03/02/2011)  
 Figure 2.3: Life cycle of *S. mansoni* in animals including Man

### 2.3.1.2 Protozoan parasites

Protozoa, like most organisms, are eukaryotic in that their genetic information is stored in chromosomes contained in a nuclear envelope. In addition, because they lead an independent existence, they possess a variety of other subcellular structures or organelles with distinct organizational features and functions. However, classification of protozoa is extremely complex such that largely the common characteristics of each group are reflected by similarities in the disease they cause (Urquhart *et al.*, 1987).

There are four phyla in the Kingdom: Protista (Animalia) for Protozoa of veterinary importance namely Sarcomastigophora, Sporozoa, Ciliophora and Microspora. Species of *Trypanosoma*, *Leishmania*, and *Giardia* belong to the subphylum Sarcomastigophora and fall under the class Mastigophora. *Cryptosporidium*, *Eimeria* and *Toxoplasma* belong to the subphylum Sporozoa and fall under the class Coccidia while *Babesia* belongs to the Subphylum Sporozoa and falls under class Piroplasmidia (Urquhart *et al.*, 1987). The phylum Sporozoa is also called apicomplexa. This alternative name refers to the group's possession of an apical complex, a structure that apparently assists penetration of the host cell. The apicomplexa have complex life cycles that are characterized by three distinct processes of sporogony, Schizogony (merogony) and gametogony (Figure 2.4). Although most apicomplexa exhibit this overall general life cycle, the details can vary between species. Furthermore, the terminology used to describe these various life cycle stages vary between the species. The life cycle consists of both asexually reproducing forms and sexual stages. In monoxenous species, all three of these

processes are carried out in a single host and often in a single cell type or tissue. On the other hand, in heteroxenous species, the various processes are carried out in different hosts and generally involve different tissues



(Adapted from: <http://www.tulane.edu/~wiser/protozoology/notes/api.html> 03/02/11)  
Figure 2.4: General apicomplexan structure and life cycle

#### 2.3.1.2.1 Gastrointestinal protozoan parasites

*Cryptosporidium parvum* and *Giardia lamblia* are the GI protozoan parasites considered to be zoonotic (Bajer *et al.*, 2010). Cryptosporidiosis, caused by *Cryptosporidium parvum*, is an enterocolitis of cosmopolitan distribution. It has a wide host range and is common in young ruminants, particularly calves. However, cryptosporidiosis is also found in man and is rare in dogs. Although cryptosporidiosis is self-limiting, infections from animals pose a significant risk to immune-compromised people who may develop protracted diarrhoea and die (Juranek, 1995). On the other hand, giardiasis is a chronic intestinal protozoan infection that has a worldwide distribution in most domestic and wild mammals, many birds and people. Giardiasis is a common infection of dogs, with younger animals being more susceptible (Thompson *et al.*, 2008).

#### 2.3.1.2.2 Haemoprotozoan parasites

Several haemoprotozoan parasites such as *Trypanosomes*, *Leishmania* and *Babesia* cause morbidity and mortality in dogs and man in the tropical regions (Urquhart *et al.*, 1987).

Canine trypanosomosis is a devastating disease of dogs that can cause anaemia, infertility, abortions and death if not treated (Losos, 1972). In advanced cases of the disease, there may also be neurological changes resulting in aggressive signs, ataxia or convulsions. Some subspecies of *Trypanosoma brucei* such as *T. brucei rhodesiense* and *T. b. gambiense* are zoonotic and may cause sleeping sickness in man (Losos, 1972). *T. vivax*, *T. congolense* and *T. brucei* are the major causes of disease in ruminants while *T. congolense*, *T. brucei* and *T. evansi* cause disease in dogs (Stephen, 1970). The epidemiology of trypanosomosis varies from one locality to another and depends largely on the level of interaction between tsetse, domestic and game animals. The nature of that interaction is subject to spatial and temporal variations. Within the tsetse belts of southern Africa, four distinct epidemiological situations can be distinguished: (i) wildlife zones in Africa where livestock is absent, (ii) areas where livestock have been recently introduced into wildlife zones, (iii) areas where livestock are present at the edge of wildlife zones (interfaces) and (iv) areas where livestock are kept in tsetse-infested zones and where large game animals are absent (Van den Bossche, 2001). Areas where livestock are kept in a tsetse-infested zone and where livestock constitutes the major host of tsetse are of particular economic importance. Such an epidemiological circumstance is usually the consequence of the gradual encroachment of people and their livestock into tsetse-infested areas and the subsequent disappearance of large game animals because of human interference and the clearing of vegetation for cultivation. It is found in large parts of the fertile and cultivated areas of Southern Africa such as the plateau of the Eastern Province of Zambia. Since the mid-1940s, the plateau of eastern Zambia has

been subject to human encroachment and large parts are currently cultivated. Cattle, goats and pigs are the main livestock species present. Game animals are scarce. *Glossina morsitans morsitans*, the only tsetse species present, is highly dependent on livestock for its survival (Van den Bossche and Staak, 1997).

Babesiosis is a tick-borne disease affecting humans and many domestic and wild animals. Dogs are susceptible to several *Babesia* species including *B. gibsoni* and *B. canis* (transmitted by Ixodes ticks). Domestic animals showing appreciable morbidity and mortality include dogs, cats, cattle and horses. Canine babesiosis is a disease characterised by haemolytic anaemia, icterus and haemoglobinuria (Schoeman, 2009). The most widespread and pathogenic species is the large *Babesia canis* found in mainland Europe, Africa, Asia and America (Urquhart *et al.*, 1987). *Rhipicephalus sanguineus* is the principal vector, in which transovarian followed by transtadial transmission occurs. Dogs may also harbour zoonotic *Babesia* species such as *B. divergens* and *B. microti*, characterised in humans by nausea, weight loss, anaemia, fever and general malaise (Gray, 2006).

Several *Leishmania* species including *Leishmania donovani*, cause disease in dogs, characterised by skin lesions, anaemia, lymphadenopathy, diarrhoea and emaciation. *Leishmania* parasites are transmitted through sand fly bite during a blood meal from infected domestic or wildlife animals (Morosetti *et al.*, 2009). The disease occurs in Southern Asia, South America and Africa and is transmissible to humans, causing debilitating and fatal conditions (Susan and Aiello, 1998).

#### 2.3.1.2.3 Effects of endoparasites on blood parameters

Endoparasitism is a widely recognized disease of dogs. Unfortunately, many of the dogs that are infected with GI helminths do not show obvious clinical signs of the disease. For such dogs, clinical laboratory examination of faecal samples is the only method of diagnosis. The effects of specific GI parasites of dogs on the haemogram of such infected dogs have been reported (Ogunkoya *et al.*, 2006; Qadir *et al.*, 2010) and these include a significant reduction in levels of haemoglobin, PCV and total erythrocyte counts, leukocytosis and eosinophilia in infected dogs (Qadir *et al.*, 2010). Canine babesiosis is mainly characterized by haemolytic anaemia, icterus and haemoglobinuria (Schoeman, 2009) which is seen on blood examination as a reduction in the PCV, haemoglobin and total erythrocyte count (Ayoob *et al.*, 2010).

#### **2.4 Laboratory diagnosis of dog endoparasites**

There are various techniques used in the laboratory diagnosis of canine endoparasites. These techniques generally depend on the type of parasite suspected in the clinical specimens.

Gastrointestinal parasites may be diagnosed grossly for the presence of gravid segments of tapeworms or microscopically through faecal examination by (i) direct smear method, (ii) flotation methods, and (iii) Ziehl-Nielsen technique (Clarke and McIntyre, 2001). It is possible to detect most eggs or larvae by the direct smear method, but due to small amounts of faeces used, it may only detect relative heavy infections (Ravel, 1995). This technique is used also to detect motile parasite stages such as protozoan trophozoites and helminth larvae frequently passed in the semi-formed and loose to fluid faeces of dogs. Flotation concentration procedures have the

potential to distort the delicate structures of these organisms and obliterate their diagnostic attributes so that accurate identification is difficult. Faecal flotation methods separate the diagnostic products of endoparasitic organisms (eggs, larvae, oocysts, and cysts) in the faeces of dogs by use of suspension medium with a higher or lower specific gravity than the parasite products. Parasite eggs, cysts, and oocysts are concentrated on the surface or bottom of the medium because of their lighter or heavier density respectively. The advantage with this technique is that you get a clean preparation for microscopic examination with a minimal amount of distracting faecal debris. The simple flotation (SF) and centrifugal flotation (CF) technique are widely used for the detection of nematode and cestode eggs. SF provides good results among other flotation technique and is one of the easiest and short ways for identifying and counting eggs. With the CF technique, more time is saved and greater accuracy obtained.

Examination of the blood by light microscopy is the most readily applied method for diagnosis of haemoparasites and, more importantly, is a technique that can be easily applied in the field. The basic parasitological technique for diagnosis of trypanosomosis has been modified to improve diagnostic sensitivity by concentrating the blood through centrifugation in a haematocrit tube, namely the haematocrit centrifuge technique (HCT) or the dark ground buffy coat technique (DG) (Paris *et al.*, 1982). Xenodiagnosis can also be used in which freshly collected blood is inoculated into laboratory rodents, which can then be examined for periods of 30 to 60 days to determine if they have developed trypanosome infections.

While clinical signs and lesions often clearly point toward a diagnosis of babesiosis, specific diagnosis depends on the detection of the parasites in the red blood cells (Ayooob *et al.*, 2010) using thin blood smears stained with Romanowsky dyes, such as Giemsa and examined under an oil immersion lens (Dacie, 1984). A number of serological tests are available for the detection of carrier animals with the most commonly used being the indirect fluorescent antibody test and enzyme linked immunosorbent assay (ELISA) (Ayooob *et al.*, 2010).

Serological (e.g. ELISA) and molecular (e.g. Polymerase chain reaction [PCR] and loop mediated isothermal amplification [LAMP]) tests are also available for diagnoses of some endoparasites but for the purpose of the thesis, these are not discussed here.

## **2.5 Treatment of dog endoparasitoses**

### **2.5.1 Gastrointestinal helminths**

While intestinal parasites usually cause less problems in young adult and adult animals, these animals can develop patent infections and are capable of contaminating the environment. Therefore, they should be regularly monitored or treated for intestinal parasite infections. While all adult animals are at risk, those that are allowed to roam or spend most of their time outside run a greater risk of becoming infected. There are a variety of anthelmintic drugs available that are safe and effective against ascarids, hookworms, and other intestinal helminths of dogs. Mature dogs should be monitored through biannual or yearly diagnostic stool examinations and treated with anthelmintic directed at specific intestinal helminths. This is because the mature adult

dogs are more resistant to parasitic infections than puppies, pregnant and nursing animals (Lorenzini *et al.*, 2007).

Doramectin is the current drug of choice in the treatment of Spirocerosis, which effectively kills adult worms, and decreases egg shedding (Van der Merwe *et al.*, 2007). A study on the prophylactic effect of doramectin on *S. lupi* in dogs had showed that although doramectin did not entirely prevent canine spirocerosis, it reduced the clinical signs associated with infection, and delayed and reduced egg output (Lavy *et al.*, 2003).

### **2.5.2 Gastrointestinal protozoan parasites**

Protozoan GI parasites that infect dogs include *Giardia* and *Cryptosporidium* (Fontanarrosa *et al.*, 2006)). There is no current approved treatment for giardiasis and cryptosporidiosis in animals. There are a limited number of drugs available for the treatment of giardiasis in domestic animals. Metronidazole, tinidazole, quinacrine and furazolidone are effective, but are avoided because they are carcinogenic and mutagenic. Benzimidazoles, e.g. fenbendazole (given at 50mg/kg/day *per os*) have been shown to effectively remove *Giardia* from the faeces of dogs (Susan and Aiello, 1998). There is currently no satisfactory chemotherapeutic agent available for the treatment of cryptosporidiosis (Juranek, 1995). Many agents have been tested *in vitro* and *in vivo*, but few agents have shown promise (O'Handley *et al.*, 1997). Of those that have been tested, paramomycin is expensive and halfuginone and lasalocid are highly toxic at the effective doses.

### **2.5.3 Haemoprotozoan parasites**

The use of drugs for the prevention and treatment of trypanosomosis has been in existence for many decades, but the rapidity with which the trypanosomes have developed resistance to each drug introduced has tremendously complicated this approach to controlling the disease. The current drugs used as veterinary trypanocides include diminazine aceturate and isomethamidium (Antia *et al.*, 2009). These are also used as prophylactics for control of the disease in cattle (WHO, 1995).

There are a number of effective babesiacides, including quinuronium sulphate, diminazene aceturate, amicarbalide, phenaminidine isethionate, and imidocarb. However, not all are available and the use of some is restricted in certain countries. Diminazene aceturate and imidocarb dipropionate are the most widely used babesiacides (Ayoob *et al.*, 2010).

## **2.6 Control of dog parasitoses**

### **2.6.1 Gastrointestinal helminths**

Most cases of human ascarid and hookworm infections can be prevented by practicing good personal hygiene, eliminating intestinal parasites from pets through regular deworming, and making potentially contaminated environments, such as unprotected sand boxes, off limits to children (Kazacos, 2000). It is also important to clean up pet faeces on a regular basis to remove potentially infective eggs before they become disseminated in the environment via rain, insects, or the active migration of the larvae (Samuel *et al.*, 2001).

### **2.6.2 Gastrointestinal protozoan parasites**

Cryptosporidiosis is difficult to control since the oocysts are highly resistant to most disinfectants (Garcia, 2007) except formalin and ammonia (Urquhart *et al.*, 1987). *Giardia* cysts are immediately infective when passed in the faeces and survive in the environment. Prompt removal of faeces from cages, runs and yards limits environmental contamination.

### **2.6.3 Haemoprotozoan parasites**

The control of trypanosomosis depends on several levels, including eradication of tsetse flies and use of prophylactic drugs. Tsetse flies can be partially controlled by frequent spraying and dipping of animals, spraying of insecticides on fly-breeding areas, use of insecticide-impregnated screens, bush clearing, and other methods. Dogs can be given drugs prophylactically in areas with a high population of trypanosome-infected tsetse (Antia *et al.*, 2009).

The most effective preventative strategy of babesiosis is control of the tick vector, *R. sanguineus* (Ayoob *et al.*, 2010). Frequent visual inspection of the skin and hair coat is an effective means of tick control as a minimum of two to three days of tick engorgement is necessary for parasite transmission. Visual inspection should be combined with topical acaricide therapy to prevent tick infestation. Topical therapies of proven benefit include amitraz impregnated collars fipronil and imidaclopridpermethrin applied once monthly, all resulting in prevention of tick attachment or tick death within 24 to 48 hours (Ayoob *et al.*, 2010). Vaccination using live, attenuated strains of the parasite has been used with some success in a number of countries, particularly Australia and South Africa (Schetters *et al.*, 1997).

## **2.7 Important findings in global context**

Public-health problems caused by the impact of dogs on humans are both direct and indirect (Baxter, 1984). Many canine gastrointestinal parasites eliminate their dispersion elements (eggs, larvae, oocysts) by the faecal route. Public sites such as playgrounds, parks, gardens, public squares and sandpits may be an important source of human infection (Rubel and Wisnivesky, 2005). Borg and Woodruff (1975) described some of the medical problems encountered in humans following ingestion of *Toxocara canis*: endophthalmitis, myocarditis, epilepsy, hepatomegaly, asthma, pneumonitis and eosinophilia.

Studies on canine endoparasites have indicated different prevalences of the common parasites found in dogs and the risk that these diseases pose to the public has well been established and documented (Fontanarrosa *et al.*, 2006). Furthermore, differences of parasite burden in different age groups and sex have also been demonstrated by some researchers (Gates and Nolan, 2009; Bridger and Whitney, 2009). The effects of GI helminthosis on the haemogram of dogs have also been demonstrated and documented by some researchers (Ogunkoya *et al.*, 2006; Qadir *et al.*, 2010).

A 1967 study reported that, in Mexico City, stool samples of 93 percent of 120 stray dogs below six months of age contained *T. canis* (Styles, 1967) whereas in 1970 about 20 percent of dogs in Britain had *T. canis* ova in their stools and this figure was similar to that from some parts of the US (Borg and Woodruff, 1975). A study in Montreal demonstrated a prevalence of 43.5 percent of *T. canis* in 239 stray dogs (Seah *et al.*, 1975). The prevalence of gastrointestinal parasites in a study by Bugg *et*

*al.* (1999) of a sample of urban dogs in Australia originating from five sources demonstrated a prevalence of gastrointestinal parasitism higher in pet shop puppies (51 percent) than in dogs from refuges (37 percent), breeding kennels (32.7 percent), veterinary clinics (15.6 percent) and exercise areas (5.3 percent). The study also found that puppies less than six months of age, dogs living in households with more than one dog, and dogs from refuges were significantly more likely to be parasitized. The prevalence of parasitoses in stray dogs from Mexico City that were collected and dissected by Eguia-Aguilar *et al.* (2005), revealed a prevalence of 85 percent. The cestodes collected were *D. caninum* (60 percent), *Taenia hydatigena* (2.5 percent), *Taenia pisiformis* (1.6 percent), *E. granulosus*, *Mesocestoides vogae* and *Mesocestoides variabilis* in only one animal each (0.83 percent), the latter two were collected for the first time ever in Mexico City. Nematodes collected were *A. caninum* (62.5 percent), *T. canis* (13.3 percent) and *Toxascaris leonina* (4.16 percent). By age, *D. caninum* and *A. caninum* were the most prevalent species in older animals, while *T. canis* was more prevalent in young animals. By season, *T. canis* was most common in the dry season (Eguia-Aguilar *et al.*, 2005). In Argentina, Fontanarrosa *et al.* (2006) reported overall prevalence of 52.4 percent of intestinal parasites. Species prevalence corresponded to *A. caninum* (13 percent), *Isospora ohioensis* complex (12 percent), *T. canis* (11 percent), *Trichuris vulpis* (10 percent), *Sarcocystis* sp. (10 percent), *Giardia duodenalis* (9 percent), *Isospora canis* (3 percent), *Hammondia-Neospora* complex (3 percent), *D. caninum* (18 cases), *Cryptosporidium* sp. (5 cases), and *T. leonina* (1 case). There was no significant difference in the overall prevalence between genders, and breeds, but prevalence in puppies (<1 year) was higher than in adult dogs. Only

the prevalence of *A. caninum* differed between genders, with higher values for males. The prevalences of six of the parasite species showed a decreasing trend with increasing host age, and an inverse pattern was found for two other species. The prevalences of three protozoa were significantly higher in pure-breed dogs, and those of two nematodes were significantly higher in mixed-breed dogs. Only prevalences of *Sarcocystis* sp. and *G. duodenalis* showed seasonal variation (Fontanarroza *et al.*, 2006). In Italy, Switzerland, Sager *et al* (2006) revealed the presence of helminths: *T. canis* (7.1 percent), hookworms (6.9 percent), *T. vulpis* (5.5 percent), *T. leonina* (1.3 percent), Taeniid eggs (1.3 percent), *Capillaria* spp. (0.8 percent), and *Diphyllobothrium latum* (0.4 percent) from coproscopic examination of dogs. Martinez-Moreno *et al.* (2007) had reported the prevalence of gastrointestinal parasites in dogs in Spain, with special attention to those parasites that can be transmitted to man. The overall prevalence was 71.33 percent. Parasites species prevalence of the gastrointestinal tract corresponded to *Isospora canis* (22 percent), *Isospora* (*Cystoisospora*) spp. (10.22 percent), *Sarcocystis* (2.5 percent), *Hammondial/Neospora* (1.94 percent), *Giardia canis* (1 percent), *D. caninum* (13.2 percent), *Taenia hydatigena* (7.66 percent), *Taenia pisiformis* (4 percent), *Uncinaria stenocephala* (33.27 percent), *T. leonina* (14.94 percent), *T. canis* (17.72 percent) and *T. vulpis* (1.66 percent) (Martinez-Moreno *et al.*, 2007). Palmer *et al.* (2008) reported overall prevalence of gastrointestinal parasites in Australian pet dogs originating from veterinary clinics and refuges of 23.9 percent. Overall, *Giardia* was the most prevalent parasite (9.3 percent) followed by hookworm (6.7 percent) (Palmer *et al.*, 2008). In China, a study by Dai *et al.* (2009) revealed helminths prevalence of 100 percent in

adult dogs from a total of 438 adult farm dogs slaughtered in local abattoirs and all the dogs were infected by more than one helminth species. The apparent prevalence of endoparasite infections across different age groups of dogs and cats have been reported by Gates and Nolan (2009) in the USA. Endoparasitism was found to be predominantly a disease of younger animals, with peak prevalence observed almost uniformly in dogs under six months old, with the exception of *Trichuris* with its longer pre-patent period. Furthermore, nearly 50 percent of dogs under six months old with a history of parasites were diagnosed with at least one species of parasite on subsequent fecal examination. The percentage dropped to 18.4 percent in animals aged 14 years, but again increased to 31.5 percent in animals over 10 years old (Gates and Nolan, 2009). In Argentina, Soriano *et al.* (2010) reported prevalence of parasitic agents to be 37.86 percent from dog faecal samples. A total of 15 different parasitic agents (PA) were detected, including *T. canis* (16.35 percent), Taeniid eggs. *Echinococcus* spp. (12.65 percent), *T. vulpis* (6.06 percent), *Giardia* spp. (1.29 percent), *T. leonina* (0.56 percent), *A. caninum* (0.41 percent), *D. caninum* (0.31 percent), *Diphyllobothrium* spp. (0.10 percent), among others. Prevalence of PA was slightly higher in rural (40.06 percent) than in urban (33.44 percent) locations. Distribution of groups of PA (cestodes, nematodes, and protozoa) showed statistical differences between both habitats ( $p < 0.05$ ). Prevalence of cestodes (18.18 percent) and protozoa (11.86 percent) was significantly higher in the rural environment than in urban areas and nematodes (29.10 percent) were more frequent in urban locations (Soriano *et al.*, 2010).

Canto *et al.* (2010) reported the prevalence of 72.8 percent of helminth species in stray dogs, from the capital city of the state of Queretaro, Mexico. Single infections were observed in 50.5 percent of infected dogs and 49.5 percent harboured mixed infections. Out of the 378 dogs examined, 55.2 percent presented nematodes and 48.1 percent cestodes. The prevalences for each species were, *Ancylostoma caninum* 42.9 percent, *T. canis* 15.1 percent, *Spirocerca lupi* 4.5 percent, *T. leonina* 2.3 percent, *Physaloptera praeputialis* 1.9 percent, *Dirofilaria immitis* 1.3 percent, *Oslerus osleri* 0.3 percent, *D. caninum* 44.9 percent, Taeniid eggs. 6.9 percent. There were no significant differences in prevalences observed either between female (68.5 percent) and male (76.8percent) or between young (70.6 percent) and adult (74.2 percent) animals (Canto *et al.*, 2010).

Canine babesiosis caused by *Babesia canis canis* and *Babesia canis vogeli* is known to occur in Portugal (Cardoso *et al.*, 2010). Dogs can simultaneously be or sequentially infected with multiple pathogens. A study based on means of blood smear examination, PCR and DNA nucleotide sequencing, for the presence of *Babesia* spp. and co-infecting agents *Leishmania*, *Anaplasma* / *Ehrlichia* and *Hepatozoon* was carried out in Portugal by Cardoso *et al.* (2010) from dogs clinically suspected of babesiosis. 98 percent had infection with *B. c. canis* and one with *B. c. vogeli*. Co-infections were detected in 20 percent. Eight dogs were found infected with two vector-borne agents: six with *B. c. canis* and *Leishmania infantum*; one with *B. c. canis* and *Ehrlichia canis*; and one with *B. c. canis* and *Hepatozoon canis*. Another dog was infected with three vector-borne pathogens: *B. c. vogeli*, *E. canis* and *L. infantum*. Overall, prevalences of *L. infantum* (16 percent), *E. canis* (four percent), and

*H. canis* (two percent) reported out of the 45 dogs with babesiosis. Almost 90 percent of the 45 cases of canine babesiosis were diagnosed in the colder months of October (18 percent), November (27 percent), December (20 percent), February (13 percent) and March (9 percent). Co-infections were detected in February, March, April, May, October and November. A higher sensitivity of *Babesia* spp. detection was obtained with PCR assays, compared to the observation of blood smears (Cardoso *et al.*, 2010). In South Africa, a prevalence study of *Babesia* infections by Matjila *et al.* (2004) in domestic dogs using reverse line blot hybridization and 18S sequence analysis found that out of a total of 297 blood samples, 31 (10.4 percent) were positive for *B. c. rossi*, whereas *B. c. vogeli* was detected in 13 dogs (4.4 percent) and *Babesia c. vogeli* was confirmed for the first time in domestic dogs (Matjila *et al.*, 2004).

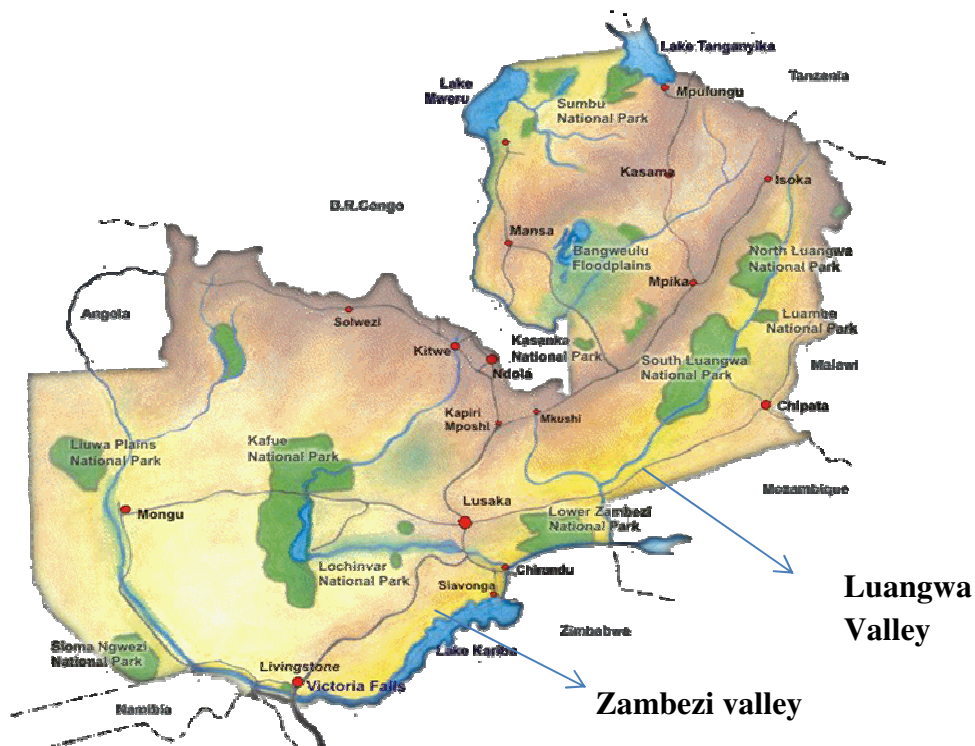
A survey of the dog and cat population in Nigeria by Okaeme (1985) revealed a significant high frequency of potential helminths of public health importance. These included hookworm *Ancylostoma* spp.; *E. granulosus*, *D. caninum* in dogs (Okaeme, 1985). Overall prevalence of 100 percent of intestinal parasites has been reported in local dogs in Congo DR by Schandevyl *et al.* (1987). The parasites species prevalence corresponded to *Ancylostoma* sp. (93.8 percent), *T. canis* (35.4 percent), *T. vulpis* (25.5 percent), *S. lupi* (14.6 percent), *T. leonina* (14.2 percent), coccidia (3.5 percent) and cestodes (2.7 percent). The prevalence of intestinal parasites was similar in both sexes. *T. canis* was more prevalent in dogs aged less than three months whereas *T. vulpis* and *S. lupi* occurred only in dogs aged over three months (Schandevyl *et al.*, 1987). In Nigeria, Okoye *et al.* (2010) reported that 52.6 percent of dogs were infected with at least one of five parasites (*Toxocara* spp., *Dipylidium caninum*, *Ancylostoma*

*caninum*, Taeniid eggs. and *Trichuris vulpis*). The prevalence of infection was comparable between the male and female dogs, but varied significantly ( $P < 0.05$ ) by age, decreasing from 78.9 percent in pups to 36.0 percent in adult dogs. The most important individual parasite infection was *Ancylostoma* spp. (39.2 percent) while *T. vulpis* was the least important (1.9 percent) (Okoye *et al.*, 2010).

In South Africa, a study by Minnaar *et al.* (2002) involving 63 stray dogs destined for euthanasia revealed *D. caninum* as being the most common helminth, and was recovered from 44 percent of dogs, followed by Taeniid eggs. (33 percent), *T. leonina* (32 percent), *A. caninum* (27 percent), *T. canis* (21 percent), *A. braziliense* (19 percent), *S. lupi* (13 percent) and *Joyeuxiella* sp. (5 percent) (Minnaar *et al.*, 2002). Overall prevalence of intestinal helminths of 68.4 percent has been reported in Nigeria by Ugbomoiko *et al.* 2008 with *T. canis*, *Ancylostoma* sp. and *T. vulpis* being the most common (prevalence 14.4 percent to 41.7 percent). Prevalence patterns in helminths were age-dependent, with *T. canis* showing a decreasing prevalence with age of host, and a reverse trend in other parasite species (Ugbomoiko *et al.*, 2008).

Cases of canine trypanosomosis have been confirmed by Keck *et al.* (2009) in military working dogs, in a cross-sectional study that was undertaken to evaluate the source of infection and determine the prevalence of canine infection with *Trypanosoma congolense* in the urban focus of Abidjan, Ivory Coast. Blood from 123 dogs were collected and subjected to PCR using specific primers for *Trypanosoma congolense* "forest type". The observed prevalence was 30.1 percent and PCR positivity to *Trypanosoma congolense* was not significantly associated with sex or age of animals (Keck *et al.*, 2009).

Although in Zambia there have been no documented cases of canine trypanosomosis, the parasite has been identified in blood samples of purebred dogs originating from Mfuwe, Luangwa, Chirundu and Siavonga (Unpublished data, University of Zambia). These dogs may act as reservoirs or carriers of the causative agent of sleeping sickness especially in the tsetse fly belt regions in Zambia (Figure 2.5).



(Sourced from: RTTCP Zimbabwe, 1995)  
 Figure 2.5: Trypanosomosis endemic areas of Zambia mainly in “common fly-belt” centered in Luangwa valley and Zambezi valley

Research on intestinal parasitism and canine *Babesia* in Zambia originating from Lusaka has been done (Islam and Chizyuka, 1983; Nalubamba *et al.*, 2011). Intestinal parasites indicated a higher prevalence of cestodes helminths than nematodes with an overall prevalence of 40 percent (Islam and Chizyuka, 1983), while the monthly prevalence rates of *Babesia* ranged from 0 to 2.4 percent in natural

population and from 0 to 28.6 percent in laboratory specimens (Nalubamba *et al.*, 2011).

GI parasites of dogs have been reported to cause leukocytosis and eosinophilia on the haemogram of such infected dogs in Nigeria by Ogunkoya *et al.* (2006). In India, a coprological examination study by Qadir *et al.* (2010) revealed a 19.5 percent overall prevalence of different species of canine helminths. The parasitized dogs had significantly lower levels of haemoglobin, packed cell volume and total erythrocyte counts than non-parasitized animals. Values of other parameters, except for lymphocytes and eosinophils, were not different between the two groups. Analyses of the haematological profile revealed normocytic hypochromic anaemia in the parasitized group of dogs (Qadir *et al.*, 2010).

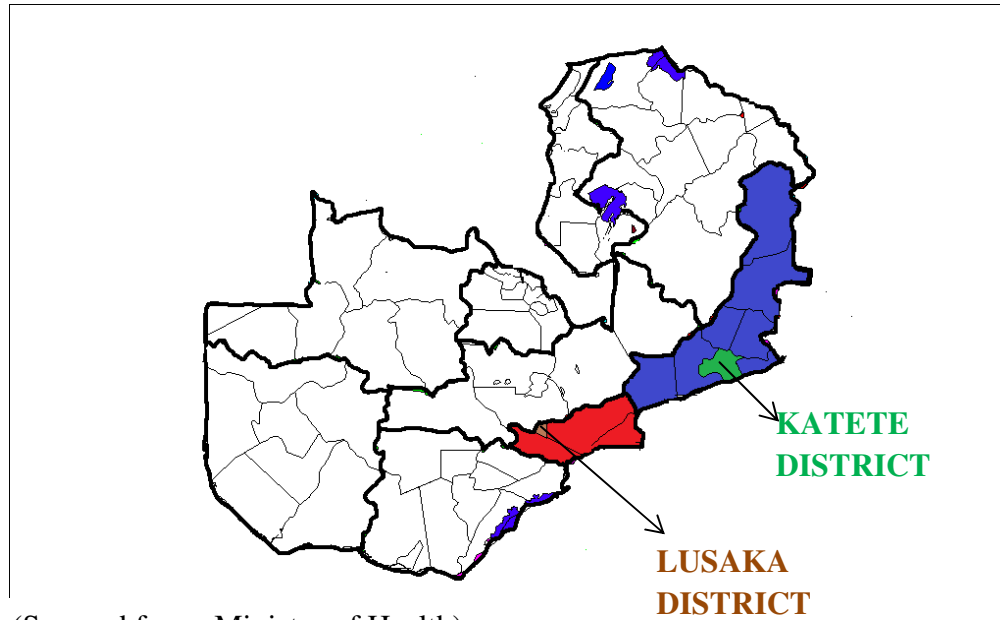
Based on the available information, it is clear that in Zambia there is paucity of information regarding the epidemiology of canine endoparasites as limited work has been done to date. The present study therefore attempted to bridge the paucity of information on the prevalence of canine endoparasites with particular regard to those with the potential to cause zoonoses and to establish some of the risk factors that may be associated with endoparasitism.

## **CHAPTER 3 METHODOLOGY**

### **3.1 Study areas**

The present study was conducted in one urban (Lusaka) and one rural (Katete) districts of Zambia (Figure 3.1). Zambia is a landlocked country located in Southern Africa and has a total area of 752 614 km<sup>2</sup>. It has a tropical climate, with three seasons: a distinct warm, wet rainy season between November and April, followed by a cooler dry season (May to July) and finally, a hot season that precedes the rainy season. Lusaka Province covers a total area of about 21,898 km<sup>2</sup> and is divided into four districts. It is the largest and capital city of Zambia. It is located in the Southern part of the central plateau, S15°25'S, 28°17'E at an elevation of 1279 m. The University of Zambia veterinary clinic, Vet Serve clinic and Lusaka Animal Welfare Society (LAWS) animal shelter within Lusaka district were randomly selected as sampling units.

On the other hand, Katete is a small, largely rural district in the Eastern Province of Zambia. It lies 11°13'S, 33°09'E at an elevation of 1060 m on the watershed between the Luangwa and the Zambezi rivers. The Eastern Province covers 69,000 square kilometers, about 9 percent of Zambia's total area, and is divided into eight districts. The plateau of the Eastern Province has a flat to gently rolling landscape with altitudes ranging from 900 to 1200 m. Sampling was undertaken in Katete district. Two rural veterinary camps namely Mthunya and Chipopela were conveniently selected as the sampling units in Katete based on easier accessibility and proximity to the district veterinary office laboratory.



(Sourced from: Ministry of Health)

Figure 3.1: Map of Zambia showing the two districts (Lusaka and Katete) where the sampling was done

### **3.2 Study design**

A cross sectional study was conducted from January 2010 through December 2010 to determine the prevalence of canine endoparasites infestation in dogs with particular regard to those with the potential to cause zoonoses and to establish some of the risk factors that may be associated with endoparasitism in urban Lusaka and rural Katete districts of Zambia. Lusaka and Katete districts were conveniently selected as the study areas based on accessibility and proximity to laboratories. The register for all veterinary clinics in Lusaka was obtained from the registrar of companies. Only veterinary clinics with a record of vaccinating above 500 dogs a year and one animal welfare shelter were included in the study. Simple random sampling was used to draw five sampling units from the sampling frame. A computer generated simple random sampling formula in Microsoft Excel® was used.

$$F = \text{rand} () * (N - n) + n$$

Where F = number of the corresponding name of veterinary clinic

N= total number of registered veterinary clinics

n= number of veterinary clinics were samples will be collected

Four veterinary clinics, namely University of Zambia veterinary clinic (UNZAVET), Showground veterinary clinic, Petvet clinic and Vet Serve clinic, and Lusaka Animal Welfare Society (LAWS), were randomly selected as the sampling units for Lusaka district. However, due to resource constraints, sampling was only conducted at UNZAVET, Vet serve and LAWS.

In Katete district, the sampling sites were selected conveniently. Informed consent to participate in the study was obtained from the dog owners.

### **3.3 Sample size**

Based on expert advice and previous studies in the areas, the prevalence of canine endoparasites was assumed to be 50 percent. The sample size to estimate the prevalence of canine endoparasitoses in dogs was calculated as described by Dohoo *et al.* (2003) using the formula:

$$n = \frac{Z^2 \times P \times Q}{L^2}$$

Where: n = required sample size, Z = Z value for a given confidence level, P = known or estimated prevalence, Q = (1-P), and L = allowable error. In this study a 95 percent confidence level with allowable error of estimation of 0.05 was used. P was estimated at 50 percent to give the maximum sample size. Therefore,  $n = 1.96^2 \times 0.5 \times 0.5 / 0.05^2 = 384$ . Thus, at least 384 dogs were to be examined for presence/absence of endoparasites.

### **3.4 Sample collection**

Before sampling, dogs were examined clinically to determine their health status. From clinical examination, basic parameters for each subject such as the rectal temperature, presence or absence of lymphadenopathy, capillary refill time and colour of mucous membranes were determined (Annex 8.6). Breed, sex, age, body condition score and worming status were determined. A total of 486 dogs (female = 224 and male = 262) ranging from less than three months to over five years old were sampled and in addition 33 stray dogs in Lusaka (male = 18 and female = 15) destined for euthanasia were collected for necropsy.

Blood samples were aseptically collected from the cephalic vein or jugular vein using 5.0 ml syringes and 21 G needles. The blood samples were stored in well-labeled EDTA tubes and examined immediately for blood parasites or refrigerated at 2 to 8°C and examined within 24 hours. Fresh faecal samples were collected from the rectum of dogs using well-labeled latex examination gloves. A minimum of 5 grams of faeces was collected and stored in the refrigerator at 4°C until examination (within 24 hours of collection).

To ensure that the required sample size was obtained in Katete, a free rabies vaccination campaign was carried out. During the rabies vaccination, all dogs were clinically examined as described above. Faecal samples that were obtained from Katete were preserved by addition of twice the faecal volume of 10 percent formalin.

In addition, stray dogs in Lusaka district destined for euthanasia were collected and humanely euthanized after obtaining consent from veterinary clinics.

Gastrointestinal worms from the intestines and body tissues of stray dogs were recovered, identified and quantified.

### **3.5 Laboratory analysis**

#### **3.5.1 Blood examination**

##### **3.5.1.1 Determination of packed cell volume (PCV)**

The PCV was measured using the microhaematocrit method as described by Embert (1986). Blood was collected into heparinised capillary tubes. The blood was allowed to enter the tube by capillarity, leaving it at least 15 mm unfilled. The tube was then sealed at one end using sealant. The sealed tubes were placed in a microhaematocrit centrifuge and spun at 9 000 revolutions per minute (rpm) for five minutes before measuring the PCV using a PCV reader.

##### **3.5.1.2 Determination of total plasma protein (TPP)**

The total plasma protein (TPP) was measured using the refractometric method as described by Embert (1986). This method involved obtaining plasma from the capillary tubes after measuring the PCV. The plasma was obtained by cutting with diamond pencil at the erythrocyte-plasma junction and extruding a single drop of plasma on the prism of a hand held refractometer and viewed through the eye piece. The total protein level was read from the hand column at the point where the dividing line between dark and bright crossed the scale. The TPP was measured in mg/dl.

##### **3.5.1.3 Examination of buffy coat**

The buffy coat was examined using the wet preparation method as described by Urquhart *et al.* (1987). This involved placing on a clean glass slide the entire buffy coats from capillary tubes from which the packed cell volume (PCV) and total plasma

protein (TPP) were measured, placing a cover slip on top and examining by light microscopy for motile trypanosome. At least 50 fields were observed before declaring a slide negative.

#### 3.5.1.4 Thin smear examination

Thin smears were made as described by Dacie (1984). A drop of blood was placed in the centre line of a glass slide. Another slide, which was used as a spreader was placed at an angle of 45 degrees to the slide containing the blood drop and moved back to make contact with the drop. The drop was allowed to spread along the line of the spreader and the film was spread by a rapid, smooth, forward movement of the spreader. The smears were dried and stained with Giemsa and examined at x100 under oil immersion for the detection of haemoparasites. A minimum of 100 fields were examined to determine parasitaemia.

#### 3.5.1.5 Differential white blood cell count

The resultant stained thin smears described previously were used for differential leukocyte counts as described by Embert (1986). Stained thin blood smears were examined using the oil immersion objective for accurate cell identification. The slides were examined starting with the thin end of the smear, and systematically traversing the slide. The fields that were used for examination were those in which erythrocytes were well separated and the leukocytes thinly spread. Cells were identified according to Jain (1987) and a record was made using a multiple tally counter. A total of 100 cells were counted per slide.

### **3.5.2 Stool examination**

Faecal samples were examined macroscopically for *D. caninum* proglottids before being processed by the simple and centrifugal flotation methods, using a saturated sodium chloride/sugar solution (specific gravity 1.28) and Sheather's sucrose solution (sp.gr 1.275) (Dryden *et al.*, 2006). A dog was categorized as positive if at least one egg was seen by microscopy in one of the employed techniques. The helminth eggs were identified based on their morphology and characteristic using the laboratory key (*Investigato Coprologica animalium domesticorum/canis et felis. Janssen Pharmaceutica Ex Scientia Progressus*) and Soulsby (1982).

#### 3.5.2.1 Simple Flotation technique

About two to three grams of stool sample was taken in a beaker and 30 milliliters (ml) of sodium chloride/sugar mixture flotation fluid (sp. gr. 1.28) was added. The faeces and flotation fluid were thoroughly mixed with a mixing stick and filtered with a tea strainer. The filtered sample was poured into a test tube and placed on a test tube rack to stand. The test tube was topped with the suspension, leaving a convex meniscus at the top of the tube and a coverslip was carefully placed on top of the test tube. The test tube was then left to stand for 10-15 minutes and then the coverslip was lifted and placed on a microscope slide and examined at x10 and x40 objective lens.

#### 3.5.2.2 Centrifugal flotation technique

About two to three grams of stool sample was well mixed with 30 ml of water and strained through a tea strainer to remove the coarse faecal material. The filtered sample was poured in a test tube, centrifuged lightly at 100 rpm for two minutes, two

or three times, until the supernatant was clear. The supernatant was removed leaving only the sediment, which was mixed with 30 ml Sheather's sucrose flotation fluid (sp. gr. 1.28) and centrifuged at 500 rpm for one to two minutes. Sheather's sucrose solution was filled to the top of the test tube leaving a convex meniscus at the top of the tube and a coverslip carefully placed on top of the test tube and left to stand for three to five minutes. The coverslip was carefully lifted off the test tube, placed on a microscope slide, and examined at x10 and x40 objective lens.

### **3.5.3 Post-mortem examination**

Dogs destined for euthanasia were collected after seeking written consent from two veterinary clinics namely, UNZAVET and Vet Serve. The dogs were clinically examined and blood was collected as described in section 3.4. The subjects were humanely euthanized using intravenous pentobarbitone sodium (Euthatal®) at 100-200mg/kg. The entire gastrointestinal tract from the oesophagus to the rectum was extracted by making a midline incision from the ventral neck region to the pubic brim. This was followed by accessing the thoracic and abdominal organs *in situ* by performing a thoracotomy via the sternotomy and ventral abdominal midline laparotomy.

Recovery of worms was as described by Urquhart et al. (1987). As soon as the alimentary tract was removed from the body cavity, the stomach and duodenal junction, and ileocaecal junction were ligated to prevent transfer of parasites from one site to the other. The stomach and oesophagus, small and large intestines were separated following ligation of these segments with nylon. The oesophagus was examined by palpation for lesions of *S. lupi* before opening it all the way up to the

stomach and examined for presence of worms. The small intestine was opened along its entire length, the contents washed into a bucket, passed through a coarse mesh sieve (aperture 212  $\mu\text{m}$ ), and the residue was collected and stored in 200 ml duplicates in suitably labeled containers and preserved in 10 percent formalin. The large intestine was opened along its entire length and the contents treated as for the small intestines.

During postmortem examination, striated muscles of the diaphragm, intercostal, masseter, tongue and hamstring were collected and processed for examination for the presence of encapsulated *Trichinella* larvae. These samples were examined using the direct trichinelloscopy method by squeezing a small sample of about one gram between two glass slides and examining for the presence of coiled larvae by direct microscopic examination or using a light source (Manual of Veterinary Parasitological Techniques, 1980). However, this method has a limitation of only being able to detect high infestation and is less sensitive in detecting low to moderate infections compared to the artificial digestion method (Beck *et al.*, 2005).

Identification and counting of gastrointestinal parasites proceeded as described by Soulsby (1982) and Urquhart *et al.* (1987).

### **3.5.4 Worm counting and identification**

About two to three ml of iodine solution was added to the preserved samples in 200 ml containers. After thorough mixing, about four ml of the suspension was transferred to a petri dish, scored with lines to facilitate counting and to this two to three ml of sodium thiosulphate solution was added to decolourise the debris. The petri dish with the collected worms were then examined using  $\times 12$  objective of a stereoscopic microscope and the worms counted, identified and stored in 70 percent

ethanol. Once completed the remaining material was discarded, another small amount of mixture poured into the petri dish and the procedure repeated.

Nematodes that could not be identified from the procedure above were cleared by immersing in lactophenol on a microscope slide for a period of three hours prior to examination under a compound microscope using magnification of  $\times 10$  and  $\times 40$ . Helminths were identified using species descriptions from Soulsby (1982) and Urquhart *et al.* (1987).

### **3.6 Data handling, storage and analysis**

The data was entered into a Microsoft Excel<sup>®</sup> spreadsheet and data quality verified for entry errors, by comparing data entries with the original data forms. The data was then transferred to SPSS version 16.0 for descriptive and inferential statistics. The Chi-square test and Fisher's exact test were used to determine differences in the GI helminth and haemoparasites prevalence between Lusaka and Katete. Comparisons of the prevalence of parasites based on age and sex were assessed using Chi-square test and Fisher's exact test. The one-way analysis of variance (ANOVA) was used to determine the significance of differences in eosinophil count, PCV and TPP in the different dogs, and the age groups and overall prevalence with the presence of one helminth. Post hoc multiple comparisons using Bonferroni was used to determine differences between the age categories for each GI helminth. Significant differences were defined as those with  $p < 0.05$ .

## **CHAPTER 4 RESULTS**

### **4.1 Sample demographics**

All dogs examined appeared clinically healthy. The distribution of different types of breeds that were recruited in the study is indicated in Annex 8.1. Mongrels were the most common breed that were observed in the study with 100 percent (160/160) of the dogs sampled in Katete being mongrels compared to 86 percent (279/326) in Lusaka. The age distribution of the dogs is indicated in Annex 8.2. The age of dogs ranged from less than three months old to over five years old. The overall statistics indicated that dogs aged >12 to 24 months and >24 to 60 months were the most sampled dogs with percentages of 28.9 percent (140/485) and 26 percent (126/485) respectively. Male dogs represented 53.9 percent (262/486) of the study population while female dogs were 46.1 percent (224/286). Lymphadenopathy in all the palpable lymph nodes (Submandibular, prescapular and popliteal) was the most predominant finding (13.3 percent) followed by dogs with lymphadenopathy involving only the submandibular (12.3 percent) (Annex 8.9). Of the dogs examined, 87.9 percent (218/248) had capillary refill time of less than two seconds. In addition, 83.5 percent (207/248) of the dogs had pink and moist mucous membranes (Annex 9.0).

Whole blood and faecal samples were collected from 84 percent (408/486) of the dogs in Lusaka and Katete while whole blood only and faecal sample only were collected from 6.6 percent (32/486) and 9.5 percent (46/486) respectively. From the data obtained 100 percent (160/160) and 89.3 percent (293/326) of all the dogs sampled had no history of previous deworming in Katete and Lusaka, respectively.

The history of deworming for dogs in Lusaka was as follows: 5.2 percent (17/326) had been dewormed <3 months before being sampled, 2.5 percent (8/326) had been dewormed 3 to 6 months before being sampled, 0.6 percent (2/326) had been dewormed >6 to 12 months before being sampled and 1.8 percent (6/326) had been dewormed >12 months before being sampled. The mean differential leukocyte count for the dogs that were examined in this study is indicated in Annex 9.1.

#### **4.2 Prevalence of canine GI helminths**

In Lusaka, the prevalence of *A. caninum* in previously dewormed dogs was 41.2 percent (7/17) (Dewormed <3 months), 87.5 percent (7/8) (Dewormed 3-6 months), 100 percent (6/6) (Dewormed >6 months). The prevalence of *T. canis* in these previously dewormed dogs was 5.9 percent (1/17) (Dewormed <3 months), 12.5 percent (1/8) (Dewormed 3-6 months) and 20 percent (1/5) (Dewormed >12 months). The rest of the detected parasites were all from the dogs that had never been dewormed.

Male dogs showed a slightly higher overall prevalence of GI helminths than female dogs, 80.9 percent (196/242) versus 75.5 percent (160/212). However, the difference was not statistically significant ( $p > 0.05$ ). Of the female dogs, presence of at least one helminth parasite corresponded to 68.4 percent (145/212) with *A. caninum*, 11.8 percent (25/212) *T. canis*, 8.5 percent (18/212) *T. vulpis*, 6.6 percent (14/212) *D. caninum*, 5.7 percent (12/212) *T. leonina* and 0.9 percent (2/212) Taeniid eggs. Of the male dogs 74.8 percent (181/242) were infected with *A. caninum*, 10.7 percent (26/242) *T. canis*, 5.0 percent (12/242) *T. vulpis*, 5.8 percent (14/242) *D. caninum*, 3.3 percent (8/242) *T. leonina* and 0.4 percent (1/242) Taeniid eggs.

The overall prevalence of dogs with GI helminths was 78.4 percent (356/454) while that for presence of at least one helminth parasite corresponded to 72.2 percent; broken down as follows ÷ *A. caninum*, 11.2 percent *T. canis*, 6.6 percent *T. vulpis*, 6.2 percent *D. caninum*, 4.4 percent *T. leonina* and 0.7 percent Taeniid eggs (Table 4.1) Katete had higher prevalence of dogs with GI helminths (82.5 percent, 132/160) compared to Lusaka (76.0 percent, 222/292). Of particular note, *A. caninum* was the most prevalent intestinal helminth in both Lusaka (73 percent) and Katete (70.6 percent) (Table 1). Furthermore, the prevalence of the nematode *T. vulpis* and cestode *D. caninum* were significantly higher ( $p < 0.05$ ) in Katete (18.1 percent and 13.1 percent, respectively) than in Lusaka (0.3 percent and 2.4 percent respectively). Taeniid eggs were very low in both populations. The prevalences of *T. canis* and *T. leonina* in Lusaka were comparable to those in Katete.

Table 4.1 - Prevalence of gastrointestinal helminth infection by species in dogs in Lusaka and Katete from faecal examination by faecal flotation

Helminth species	Lusaka (n = 294)		Katete (n = 160)		Overall (n = 454)	p value
	Dogs infected	Prevalence (%)	Dogs infected	Prevalence (%)	Dogs infected (P %)	
<i>A. caninum</i>	215	73	113	70.6	328 (72.2)	0.32
<i>D. caninum</i>	7	2.4	21	13.1	28 (6.2)	<0.001*
<i>T. canis</i>	32	10.9	19	11.9	51 (11.2)	0.43
<i>T. vulpis</i>	1	0.3	29	18.1	30 (6.6)	<0.001*
<i>T. leonina</i>	14	4.8	6	3.8	20 (4.4)	0.41
Taeniid eggs	2	0.7	1	0.6	3 (0.7)	0.72

\* Significantly different  
P = Prevalence

In all the age groups, the most prevalent helminth was *A. caninum*. Furthermore, significant difference ( $p < 0.05$ ) in the overall prevalence of *A. caninum* between age groups was observed between dogs over 60 months old (95% CI of 0.40 – 0.72) and 6 to 12 months old (95% CI of 0.73 – 0.90). The prevalence of *T. canis* was higher in dogs aged >12-24 months (14.3 percent) but less in those aged >60 months (2.4 percent). The prevalence of *T. vulpis* was higher in dogs <3 months old (11.8 percent) and that of *D. caninum* was higher in those aged <3 months (11.8 percent). Interestingly, low prevalence of *T. leonina* and Taeniid eggs were observed in all age categories (Figure 4.1).

Except for *A. caninum*, which indicated significant difference ( $p < 0.05$ ) in the prevalence according to age, the rest of the GI helminths showed no significant difference in the prevalence according to age and sex.

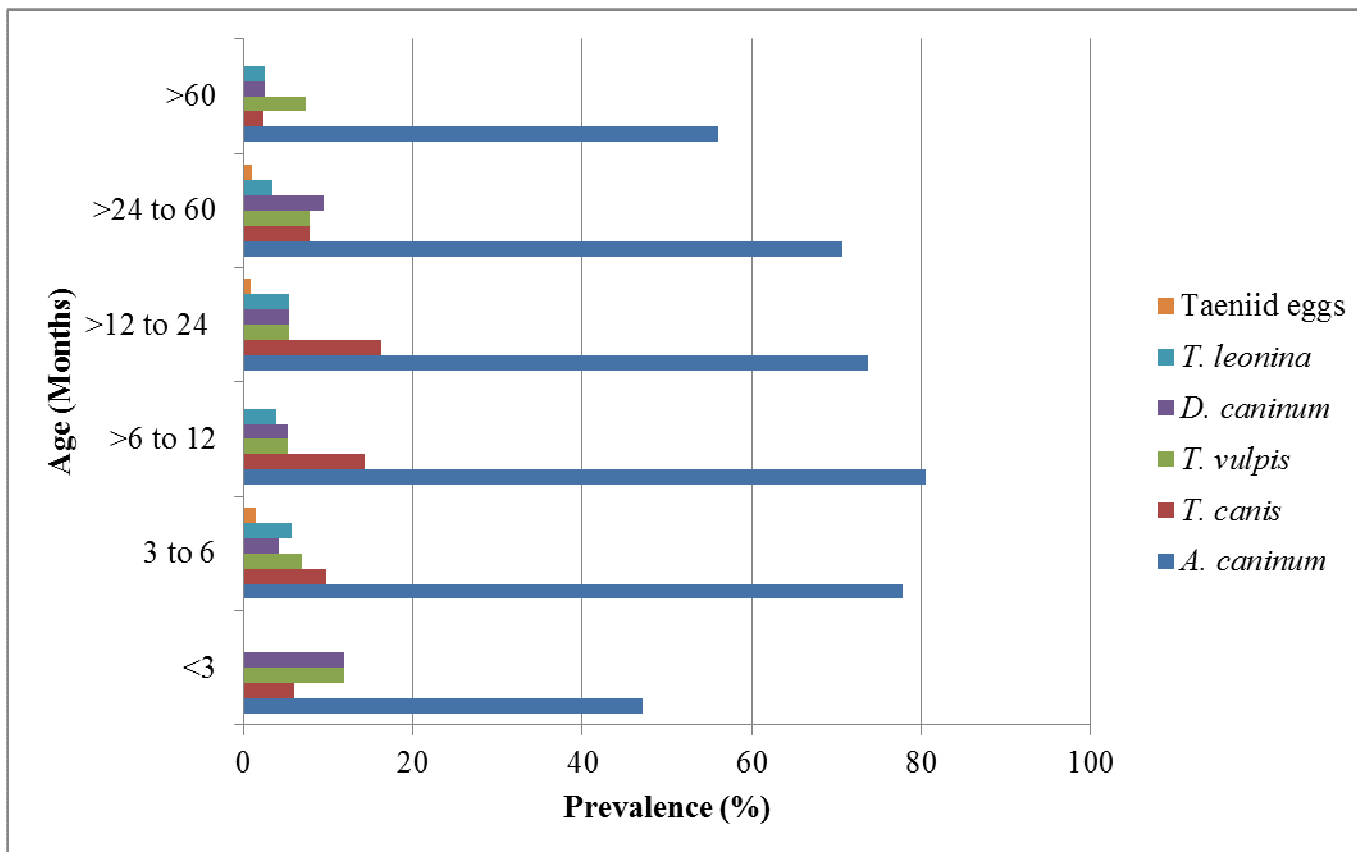


Figure 4.1: Age-specific prevalence of GI helminths ( $n = 453$ )

The prevalence of *A. caninum* single infections and mixed infections of *A. caninum* with other gastrointestinal helminths are indicated in Table 4.2 for Lusaka and Katete. Except for *A. caninum* single infections and co-infection with *D. caninum* ( $p < 0.05$ ), the prevalence of multiple infections with two and three parasites species per host was not statistically different between Lusaka and Katete. However, other distinct parasite combinations were observed between the two districts with Katete exhibiting more cases of multiple infections with two to four parasites species per host. These parasitic combinations involved *A. caninum* and *T. vulpis* 11 cases (6.9 percent); *T. vulpis* and *D. caninum* two cases (1.3 percent), *A. caninum*, *T. canis* and *T. vulpis* two cases (1.3 percent), *A. caninum*, *T. canis* and *D. caninum* one case (0.6 percent); *A. caninum*, *T. vulpis*, and *D. caninum* two cases (1.3 percent) and *A. caninum*, *T. canis*, *T. vulpis* and *D. caninum* one case (0.6 percent) whereas Lusaka showed combinations of *T. canis* and *D. caninum* two cases (0.7 percent) and *T. canis* and *T. leonina* two cases (0.7 percent)

Table 4.2 - Prevalence of single *Ancylostoma caninum* infection and co-infections of *A. caninum* with other gastrointestinal helminth species in Lusaka and Katete

<i>A. caninum</i> single & <i>A. caninum</i> mixed infections	Lusaka (n=294)		Katete (n=160)		<i>p</i> value
	Dogs positive	Prevalence (%)	Dogs positive	Prevalence (%)	
<i>A. caninum</i> single	175	59.5	69	43.1	0.01*
<i>A. Caninum</i> + <i>T. canis</i> + <i>T. leonina</i>	3	1.0	1	0.6	0.559
<i>A. caninum</i> + <i>T. vulpis</i> + <i>T. leonina</i>	1	0.3	2	1.3	0.285
<i>A. caninum</i> + Taeniid eggs	2	0.7	1	0.6	0.715
<i>A. Caninum</i> + <i>T. leonina</i>	4	1.4	2	1.3	0.643
<i>A. Caninum</i> + <i>D. caninum</i>	6	2.0	10	6.3	0.022*
<i>A. Caninum</i> + <i>T. canis</i>	24	8.2	10	6.3	0.294

\*Significantly different ( $p < 0.05$ )

### **4.3 Prevalence of canine haemoparasites**

Of the 278 dogs that were examined for blood parasites in Lusaka, only 1.1 percent (3/278) were positive for *B. canis*. Morphological characteristics of the *Babesia* in the three positive samples indicated that all were of the large-sized *B. canis* infection. Katete recorded no positive case of *B. canis*. There were no positive cases of canine trypanosomiasis reported in the blood samples using buffy coat examination and stained blood smears by microscopy.

### **4.4 Effect of endoparasitism on packed cell volume (PCV), total plasma protein (TPP) and eosinophil count.**

#### **4.4.1 The effect of endoparasitism on PCV and TPP**

Dogs, representing 84 percent of the total sampled (408/486) from which both whole blood and faecal samples were collected had their PCV and TPP determined. The districts mean PCV percent and TPP were determined to establish the effect that GI helminth co-infections had on these parameters (Annexes 8.3, 8.4 and 8.5). The mean PCV were not significantly different ( $p > 0.05$ ) with increasing number of co-infecting GI helminths except for Katete where a significant difference in mean PCV of dogs with no co-infecting GI helminths and those with two co-infecting GI helminth ( $p < 0.05$ ) was observed (Figure 4.2). Thus, there was a significant reduction in the PCV with two co-infecting GI helminths compared to those with no co-infecting GI helminths. Dogs with three co-infecting GI helminths in Lusaka had a reduced mean PCV percent but the difference was not significant from those with no co-infecting and two co-infecting GI helminths ( $p > 0.05$ ). The results for Lusaka should

however be taken with caution as only two dogs had three co-infecting GI helminths. The mean TPP in both districts was not affected by increase in number of co-infecting GI helminths (Figure 4.3). Only one dog had four co-infecting GI helminths hence was not included in the analysis.

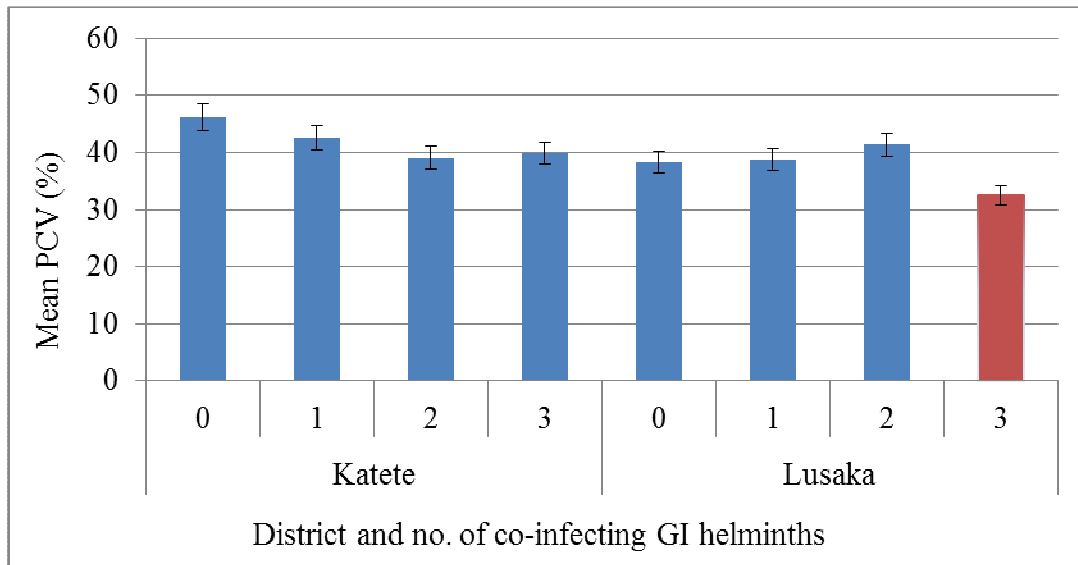


Figure 4.2: Interval plot of the districts mean PCV% values of dogs with number of GI helminths co-infections at 95% confidence interval (CI) ( $n = 408$ ).

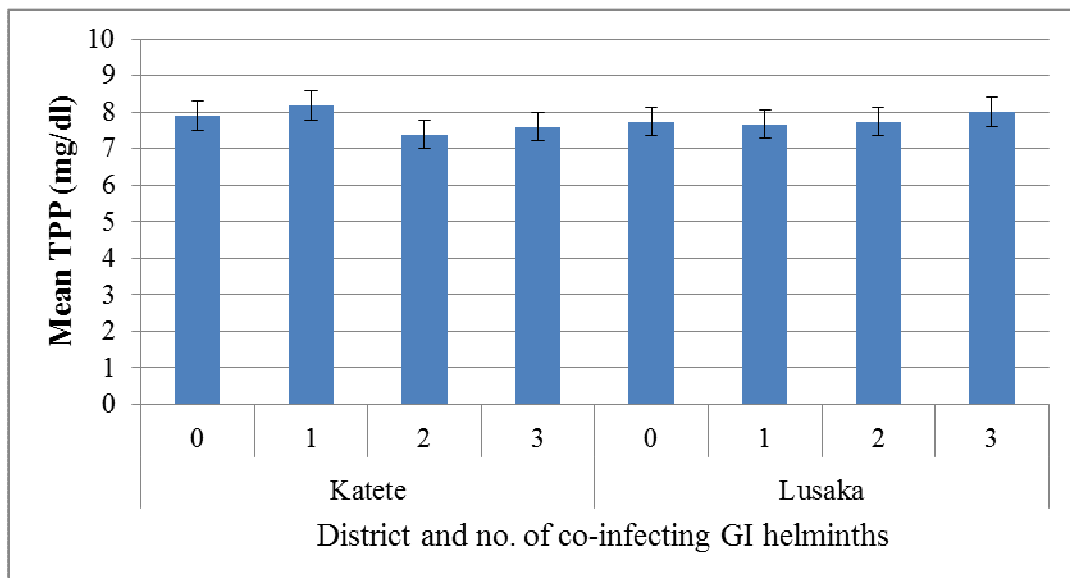


Figure 4.3: Interval plot of the districts mean TPP values of dogs with number of GI helminths co-infections at 95% CI ( $n = 408$ )

There was a significant difference ( $p < 0.05$ ) between the mean PCVs of *Babesia* positive dogs (mean 24.6 percent, range 9 to 44 percent ( $\pm$ SD = 17.8;  $n = 3$ ) and negative dogs (mean 39.1; range 12 to 61 percent ( $\pm$ SD = 8.2;  $n = 275$ ).

#### **4.4.2 The effect of GI helminths on eosinophil count**

Differential white blood cell counts were done to determine if GI helminth infestation in dogs was associated with eosinophilia. A total of 256 blood samples were examined for eosinophilia. Statistical difference ( $p < 0.05$ ) in the mean eosinophil percent between parasitized and non-parasitized dogs was observed in dogs infected with *T. leonina* and *T. vulpis* whereas no difference in the other four GI helminths was observed (Table 4.3). Only *T. leonina* demonstrated eosinophilia in dogs that were positive whereas *T. vulpis* indicated a decrease in the mean eosinophils percent in the positive dogs compared to those that were negative for the parasite. Furthermore, mean eosinophil percent for non-parasitized and GI helminths parasitized dogs did not show statistical difference ( $p > 0.05$ ) (Figure 4.4). Further assessment to determine whether GI helminth co-infections were associated with eosinophilia, revealed an increase in the mean eosinophil percent for dogs that had three co-infecting GI helminths (mean 9.6 percent, 95 percent CI for mean 2.0 – 17.1,  $n = 7$ ) than those that were non-parasitized (mean 7.7 percent, 95 percent CI for mean 6.5 – 8.8,  $n = 62$ ) (Table 4.4). The difference was however not statistically significant ( $p > 0.05$ ).

Table 4.3 – Overall mean eosinophil % values in parasitized and non-parasitized dogs for six detected GI helminth ( $n = 256$ )

<b>GI helminth</b>	<b>n</b>	<b>parasitized</b>	<b>±SD</b>	<b>non-parasitized</b>	<b>±SD</b>	<b>n</b>	<b><i>p</i> value</b>
<i>A. caninum</i>	187	7.9	5.2	7.8	4.6	69	0.94
<i>T. canis</i>	30	8.9	5.7	7.7	5.5	226	0.23
<i>D. caninum</i>	15	7.9	4.7	7.9	5.1	241	0.99
<i>T. vulpis</i>	8	3.6	1.9	8.0	5.1	248	0.015*
<i>T. leonina</i>	10	11.2	6.8	7.7	4.9	246	0.034*
Taeniid eggs	2	5	2.8	7.9	5.1	254	0.42

\* Significant different, SD- standard deviation, n – number of dogs, non-parasitized- dogs not parasitized with particular helminth species in question

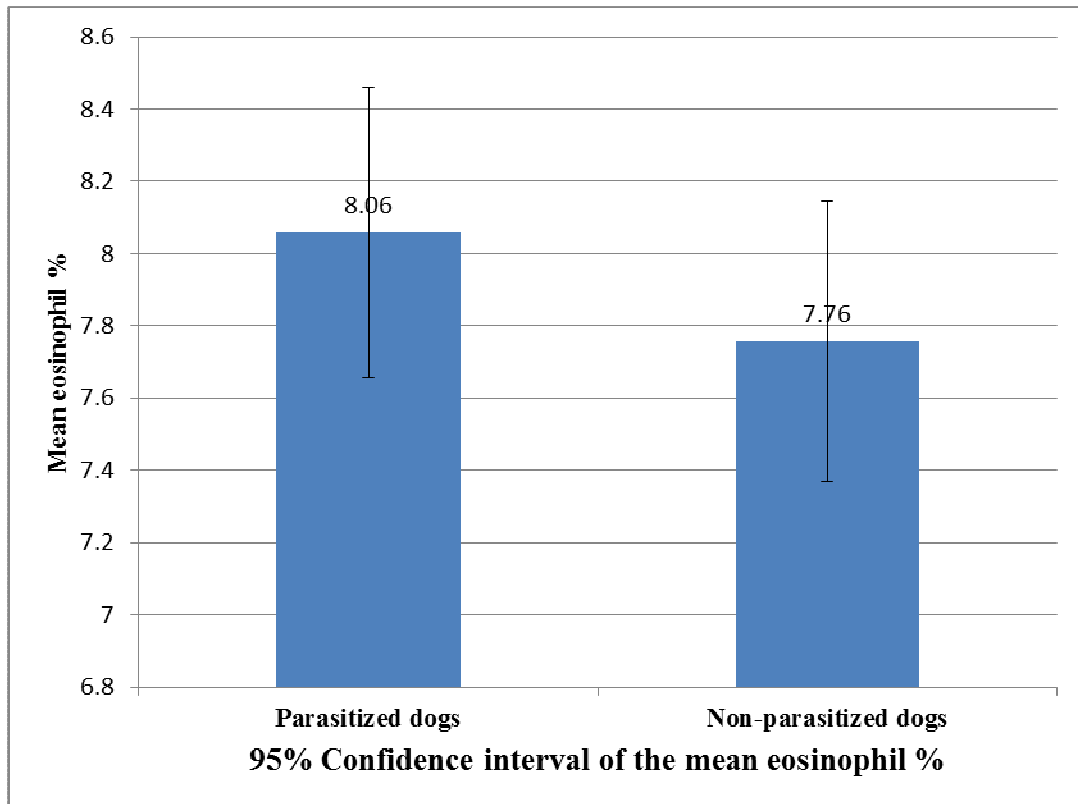


Figure 4.4: Interval plot of the mean eosinophil % of parasitized and non-parasitized dogs at 95% CI ( $n = 256$ )

Table 4.4 – Overall mean eosinophil % values of dogs and the number of co-infecting GI helminths

				<b>95% CI for Mean</b>	
<b>No. of co-infecting</b>					
<b>GI helminths</b>	<b>n</b>	<b>Mean</b>	<b>±SD</b>	<b>Lower bound</b>	<b>Upper bound</b>
0	62	7.7	4.6	6.5	8.8
1	147	7.9	5.1	7.0	8.7
2	40	8.1	4.9	6.5	9.6
3	7	9.6	8.2	2.0	17.1
4*	1	2.0	.	.	.
Total	257	7.9	5.0	7.3	8.5

\*not included in analysis because n<2, CI – confidence interval  
SD – standard deviation, n – number of dogs

#### **4.5 Prevalence of helminth parasites from postmortem studies**

Of the total dogs euthanized, 100 percent (33/33) were infected with one or more helminths parasites. Multiple infections involving nematodes and cestodes were the most prevalent followed by nematodes only with cestodes only being the least prevalent (Figure 4.5). About 64.0 percent (21/33) of dogs had multiple infections, 33.3 percent (11/33) had single infection with the nematode *A. caninum* and 3.0 percent (1/33) had single infection with the cestodes *D. caninum* (Annex 8.7 number of GI helminths species that were recovered per dog host).

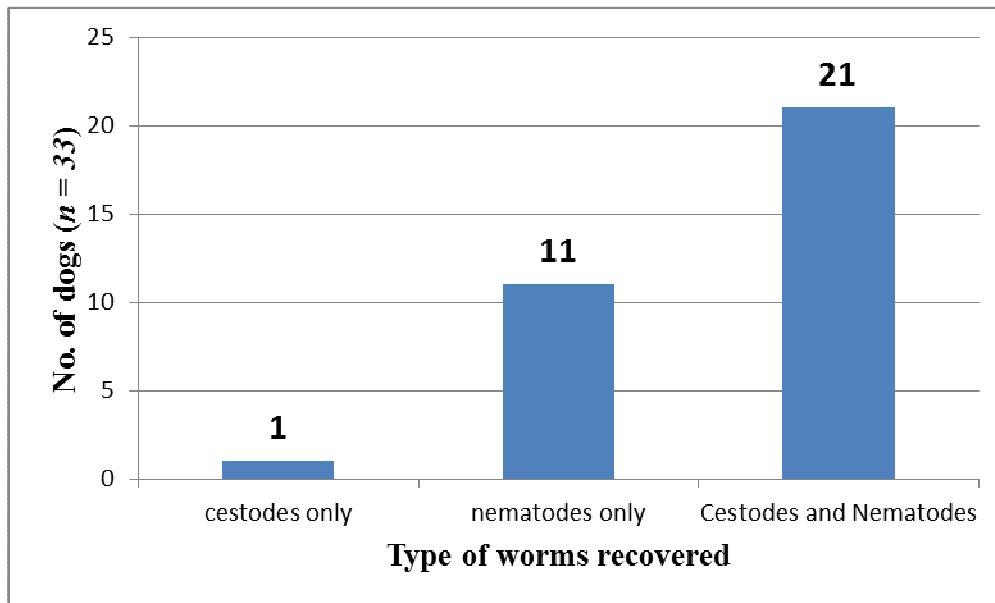


Figure 4.5: Plot of the number of dogs with single and Multiple infections from postmortem examinations ( $n = 33$ )

The prevalence of the GI helminths recovered on postmortem are presented in Table 4.5. Dogs with *D. caninum* and *S. lupi* were merely classified as being positive. The most prevalent GI helminth parasite was *A. caninum* (93.9 percent) with the least prevalent being *T. canis* (6.1 percent). The mean count per dog of *A. caninum* was

44.45 ( $\pm$ SD = 58.0) with a range of 0 to 223 (Table 4.6). There was no positive case of *T. spiralis* from the direct trichinelloscopy examination of striated muscles.

Table 4.5 - Prevalence of helminth parasites in euthanized dogs in Lusaka ( $n = 33$ )

<b>Parasite</b>	<b>No. of dogs infected</b>	<b>Prevalence (%)</b>
<i>A. caninum</i>	31	93.9
<i>D. caninum</i>	21	63.6
<i>S. lupi</i>	9	27.3
<i>T. canis</i>	2	6.1

Table 4.6 – Mean number of worms recovered per dog according to species

<b>Worm species</b>	<b>mean</b>	<b><math>\pm</math>SD</b>	<b>Range</b>
<i>A. caninum</i>	44.45	58.0	0 - 223
<i>T. canis</i>	0.33	1.74	0 - 10
<i>S. lupi</i>	-	-	-
<i>D. caninum</i>	-	-	-

#### **4.5.1 Effect of *A. caninum* burden and age on PCV**

The PCV of all euthanized dogs was measured. The results of the correlation between PCV, *A. caninum* burden and age are indicated in Table 4.7. The correlation coefficients indicated a negative correlation between *A. caninum* burden and PCV (-0.24), and age and PCV (-0.015). However, this was not a linear relationship in the reduction of PCV ( $p > 0.05$ ).

#### **4.5.2 Effect of *A. caninum* burden and age on eosinophil count**

The results of the correlation between eosinophil count, *A. caninum* count and age are indicated in Table 4.8. There was no association ( $p > 0.05$ ) between the increase in the intensity of *A. caninum* infestation and eosinophil count. Furthermore, when the effect of the other factor (age) was taken into account, *A. caninum* burdens of up to 223 did not cause a significant increase in eosinophil count of dogs.

The effect of *T. canis* burden on PCV and eosinophil count was not analyzed due to the number of dogs that were positive for the parasite being less than five. Similarly, the effect of *D. caninum* and *S. lupi* burden on PCV and eosinophil count could not be analyzed as these parasites were merely classified as being present or absent due to the difficulty in quantifying them.

Table 4.7 – The effect of *A. caninum* burden and age on PCV

		<b>PCV %</b>	<b><i>A. caninum</i></b>	<b>Age</b>
PCV %	coefficient	1	-0.24	-0.015
	<i>p value</i>		0.18	0.94
	R <sup>2</sup>		0.057	0.00
	R <sup>2</sup> (adjusted)		0.027	-0.032
	n	33	33	33

Correlation is significant at the 0.05 level (2-tailed)

Table 4.8 – The effect of *A. caninum* burden and age on eosinophil count

		<b>Eosinophil %</b>	<b><i>A. caninum</i></b>	<b>Age</b>
Eosinophil %	coefficient	1	-0.068	0.12
	<i>p value</i>		0.72	0.52
	R <sup>2</sup>		0.005	0.015
	R <sup>2</sup> (adjusted)		-0.30	-0.019
	n	33	33	33

Correlation is significant at the 0.05 level (2-tailed)

## **CHAPTER 5 DISCUSSION**

There is a dearth of information on the epizootiology of canine endoparasites in Zambia. This study provides baseline data on these endoparasites in dogs in urban and rural areas of Zambia. The most commonly sampled dogs in this study were mongrels and the age of all the dogs ranged from less than three months to dogs over five years old. This means that mongrels are the most common dogs in Zambia and because of the few numbers of exotic breeds observed in the study, the effect of breed, as a factor in endoparasitism has not been considered.

### **5.1 Prevalence of canine endoparasites from faecal flotation and blood examination diagnostic techniques.**

The overall prevalence of GI helminths in dogs from this study was higher (78.3 percent) than previously reported in Zambia (Islam and Chizyuka, 1983), in Australia (Bugg *et al.*, 1999; Palmer *et al.*, 2008), and Newfoundland (Bridger and Whitney, 2009) but similar to the findings in Spain (Martinez-Moreno *et al.*, 2007) and South Africa (Minnaar *et al.*, 2002). The higher degree of veterinary care such as regular deworming that is given to pets in Australia may explain for the difference in prevalence of GI helminth with the dogs in our study that mostly originated from resource-poor communities with little or no veterinary care given. The overall prevalence in this study was higher than the findings in Nigeria by Okoye *et al* (2010). The fact that the study by Okoye *et al.* (2010) was focussed on the epidemiology of intestinal parasites in stray dogs compared to the dogs in this study which were owned shows the low extent to which dogs in Zambia receive care in terms of feeding and proper medical attention, especially regular deworming.

Unlike findings documented by Gates and Nolan (2009) in USA, our study showed a higher overall prevalence of *A. caninum* with age from less than 3 months to 12 months old, with peak prevalence observed in dogs aged >6 to 12 months (80.5 percent). The prevalence reduced to 55 percent in dogs over 60 months old. Dogs aged 6 to 12 months had a higher prevalence of *A. caninum* compared to dogs over 5 years old ( $p < 0.05$ ). This is an important finding in that it highlights the non-existence of pet care with regard to controlling helminths via regular deworming and the level of environmental contamination with infective eggs of *A. caninum* by all the age categories of dogs. Furthermore, in agreement with Minnaar *et al.* (2002) (South Africa), Fontanarrosa *et al.* (2006) (Argentina), Okoye *et al.* (2010) (Nigeria) and Schandevyl *et al.* (1987) (Congo DR), this study equally did not show significant difference in the overall prevalence between male and female dogs in GI helminth infections.

The GI helminth species most frequently identified in this study was the nematode *A. caninum*. This is in contrast with previous reports by Islam and Chizyuka (1983) who found a higher prevalence of cestodes *D. caninum* and *T. hydatigena*. The finding of a higher prevalence of *A. caninum* in the dog population is generally in agreement with findings by other researchers (Schandevyl *et al.*, 1987; Fontanarrosa *et al.*, 2006; Okoye *et al.*, 2010). The prevalence of *A. caninum* was higher than the finding in South Africa by Minnaar *et al.* (2002). The prevalence of *A. caninum* and *T. canis* in this study were higher than the findings in Switzerland by Sager *et al.* (2006) but less than the findings in Congo DR by Schandevyl *et al.* (1987). The difference in the prevalence could be attributed to the fact that more dogs in the study in

Switzerland had been previously dewormed compared to our situation where 92.7 percent of the overall dog population in the study had never been dewormed. Furthermore, dogs that were sampled in Congo DR could have had a higher prevalence because they were less cared for and most likely had no history of deworming compared to our situation. The higher *T. canis* prevalence in adult dogs i.e. above six months was unusual since these are expected to have acquired age-dependent immunity against the helminth (Soulsby, 1982; Ugbomoiko *et al.*, 2008). The high *T. canis* infection in puppies is linked with the lifecycle of the parasite, which involves prenatal and transmission through colostrum, while resistance develops to the parasites in older dogs (Soulsby, 1982). The increased prevalence in older dogs could be attributed to the helminth species, which were not transmitted to dogs at an early age and thus did not elicit a specific immune response (Ugbomoiko *et al.*, 2008). This observation has public health implications since *A. caninum* and *T. canis* are known to cause cutaneous larval migrans and visceral larval migrans in humans respectively (Ugbomoiko *et al.*, 2008). Interestingly, our study showed a lower prevalence of *D. caninum*, Taeniid eggs and *T. leonina* than a study in South Africa by Minnaar *et al.* (2002). However, no immediate reason could be attributed to this finding and this remains an area for future research. Furthermore, unlike the findings by Bridger and Whitney (2009) in Newfoundland, our study did not show significant difference in the overall prevalence of *T. canis* between sexes. In both Zambia and Australia, the prevalence of Taeniid eggs was very low. The diagnostic technique of helminths used in this study, based on the morphological characteristics of ova as observed under the light microscope has the disadvantage that it fails to

distinguish *E. granulosus* from *Taenia* species (Ugbomoiko *et al.*, 2008). Therefore, the presence of *E. granulosus* amongst dogs with Taeniid eggs in their faeces cannot be ruled out since this helminth has been previously reported in dogs in Zambia (Islam and Chizyuka, 1983). Interestingly, cases of *A. caninum* and *T. canis* were reported in Lusaka district even in dogs that had a history of previous deworming. The prevalence of *A. caninum* appeared to be increasing with increasing time from previous deworming i.e. dogs that had been dewormed <3 months prior to sampling had a prevalence of 41.2 percent compared to dogs that were dewormed >12 months prior to sampling which recorded a prevalence of 100 percent. However, the prevalence of 41.2 percent of GI helminth in dogs that had recently been dewormed was unusual and this could be attributed to the ineffective deworming practices (ineffective anthelmintic, inaccurate doses etc.) or high re-infective pressure due to high helminth burden or resistance to the drugs used. The prevalence of *D. caninum* and *T. vulpis* were statistically higher in Katete than Lusaka ( $p < 0.05$ ). The finding of a high prevalence of *D. caninum* in rural dogs than urban is similar to the findings in Nigeria by Ugbomoiko *et al.* (2008) and this is speculated to be due to a higher flea burden of rural dogs since these are very unlikely to receive prophylactic therapeutic ectoparasites treatment than urban dogs. The significance of a high prevalence of *T. vulpis* in Katete than Lusaka in this study could not be explained and thus remains a course for further research. There was no significant difference in the other helminths between the two dog populations and this signifies the lack of routine deworming of dogs in both areas. The comparable prevalence rates of the GI helminths in the two dog populations separated by over 400km raises two vital epidemiological points.

Firstly, it suggests that the diseases and risk factors driving them are equally widely distributed in Eastern and Lusaka provinces of Zambia. Secondly, it points to a high risk of infection in human populations since some of the reported GI helminths are zoonotic (Ugbomoiko *et al.*, 2008).

The overall prevalence of *T. leonina* reported in this study was lower than in Congo DR and South Africa (Schandevyl *et al.*, 1987; Minnaar *et al.*, 2002 ) but similar to the findings in Mexico (Eguia-Aguilar *et al.*, 2005). The significance of the low prevalence of *T. leonina* in Zambia than in Congo DR and South Africa is another area for further research. There was no statistical difference in the prevalence of *T. leonina* between Katete and Lusaka.

Unlike the findings by Schandevyl *et al.* (1987), this study did not detect eggs of *S. lupi* from faecal flotation. Faecal samples sometimes require repeated examinations when the first examination is negative for *S. lupi* since the shedding of eggs is often intermittent (Dvir *et al.*, 2010). In this study, each dog was only sampled once. The other reason for *S. lupi* not being detected in faecal samples could be attributed to the fact that eggs of *S. lupi* are not found in the faeces of dogs with adult infections where the granuloma have no openings into the oesophageal lumen (Lobetti, 2000; Dvir *et al.*, 2010). This could have been the case with the dogs that were sampled for faecal GI helminth eggs. The parasite was however, recovered in the post-mortem examination of euthanized dogs from this study and was previously reported in dogs that had been euthanized in Zambia (Islam and Chizyuka, 1983).

The results of the study based on faecal examination also indicate a higher prevalence of dogs with single helminth infection than multiple infections and this is

similar to previous reports (Fontanarrosa *et al.*, 2006; Lorenzini *et al.*, 2007; Ugbomoiko *et al.*, 2008). This could be attributed to the interaction among species that depend on parasite burden rather than on the mere presence of other species (Fontanarrosa *et al.*, 2006).

The prevalence of canine babesiosis was 1.1 percent in Lusaka but undetected in Katete. This prevalence reported in Lusaka was similar to that reported by Nalubamba *et al.* (2011) but lower than that reported in Pakistan (2.62 percent) (Bashir *et al.*, 2009) and Nigeria (10.6 percent) (Amuta *et al.*, 2010). Our reported low prevalence could be attributed to a number of factors, which includes, breed, climatic conditions, clinical versus normal healthy dogs and clinical stage of the disease. Johnston *et al.* (1978) reported that non-specific or innate factors (genetic or age) possessed by the hosts could act as natural protective elements against *Babesia* and this could hold true in our situation where many of our local dogs were infested with ticks. The findings of a higher prevalence of *Babesia* in Nigeria may be attributed to climatic differences, with Zambia having a warm, dry climate than Nigeria's warmer and wetter climate (Nalubamba *et al.*, 2011). The low prevalence of babesiosis in this study could also be attributed to the fact that the sampled dogs in this study were clinically normal healthy dogs and as such, the probability of detecting positive cases was lower than would be expected if the study design were focused on detecting disease in dogs presented with suspected babesiosis. This is in agreement with the findings by Nalubamba *et al.* (2011), who found a high prevalence of *Babesia* in clinical blood samples than the normal/healthy dogs. It is also possible we could have had chronic cases of the disease even though the chronic form is uncommon, affected

dogs may be anemic but not severely, and examination of the blood from these dogs usually does not detect the parasite (Amuta *et al.*, 2010). In peracute or chronic infections, asymptomatic carriers and patients with circulatory compromise of *Babesia* cases, there is a low parasitaemia of less than 5 percent and this makes detection of the parasite via stained blood smears in red cells difficult (Ayoob *et al.*, 2010). In line with the shortcomings that have been observed with the examination of blood smears for the detection of *Babesia*, more sensitive tests (e.g. PCR and ELISA) should have been undertaken in order to get true prevalence (Nalubamba *et al.*, 2011). Dogs that were positive for *Babesia* had a significant reduction in PCV ( $p < 0.05$ ) compared to those that were negative and this is in agreement with the pathogenesis of the disease in which anaemia is a common feature (Cardoso *et al.*, 2010).

The fact that this study did not detect any positive case of canine trypanosomosis especially in the tsetse-infested Katete district should not rule out the possibility of the disease occurring in the canine population as this condition has been previously diagnosed at UNZA laboratory in purebred dogs originating from the tsetse-infested regions of Zambia (Dr. Boniface Namangala, Unpublished data) and elsewhere (Keck *et al.*, 2009). This finding may imply that the prevalence of the disease or possibly parasitaemia in Zambian dogs is generally very low. *Glossina morsitans morsitans*, the vector of trypanosomosis, takes 75 percent proportion of its meals from cattle (Van den Bossche and Staak, 1997) and such a high preference for livestock could further explain the observed results (i.e. the dog is likely to be unattractive source of feeding for *Glossina* spp) as was evidenced by previous reports on the plateau of eastern province where a low prevalence was found in goats and pigs

(Simuokoko *et al.*, 2007). Low sensitivity of microscopic diagnostic methods that were used in this study compared to the more sensitive molecular tests in the diagnosis of trypanosomosis has been documented (Simukoko *et al.*, 2007) and could partially explain the obtained results particularly in tsetse infested Katete district. According to the study that was conducted by Van den Bossche and De Deken (2002) on seasonal variation in the distribution and abundance of the tsetse fly in eastern Zambia, an apparent abundance of tsetse in miombo increased at the beginning of the rainy season (November), reached its peak at the end of the rainy season (April) and was low during the cold season (May to late August), but was lower especially in the hot dry season (September to late October). The fact that the sampling in this study was done in late August 2010 could also possibly explain results from this study as the population of the vector had declined. However, dogs are susceptible to *T. brucei* and *T. congolense* and the disease is usually acute, and apart from signs of fever, anaemia and myocarditis, corneal opacity is often a feature. There may also be neurological changes resulting in aggressive signs, ataxia or convulsions. Trypanonotolerance has been described in wildlife and to some extent in certain breed of cattle where the animal hosts are parasitaemic for long periods, but generally remain in good health (Naessens, 2006). Whether the local dogs in Zambia are resistant to trypanosomosis remains a matter of speculation. However, there is need to conduct further studies using more sensitive methods such as species-specific DNA probes or antigen-detecting ELISA.

## **5.2 Effect of endoparasitism on packed cell volume (PCV), total plasma protein (TPP) and eosinophil count.**

No GI helminth co-infections were associated with significant reductions in PCV and TPP in dogs except for Katete district where two co-infecting GI helminths had caused a significant reduction in the PCV ( $p < 0.05$ ). An acute normocytic, normochromic anaemia followed by hypochromic, microcytic anaemia in young puppies is the characteristic and often fatal clinical manifestation of *A. caninum* infection (Susan and Aiello, 1998; Lefkaditis, 2001). Surprisingly, single and mixed infections involving *A. caninum* and other GI helminths were not associated with significant reductions in PCV and this is similar to the findings in Nigeria by Ogunkoya *et al.* (2006). This could be because in older dogs, which were the most sampled, the gradual development of age resistance makes clinical disease less likely, particularly in dogs reared in endemic areas whose age resistance is reinforced by acquired immunity (Lefkaditis, 2001). Furthermore, most of the dogs that were sampled were clinically healthy dogs as compared to those that were sampled in Nigeria. This finding is very important in the epidemiology of these GI helminth infection as these dogs act as reservoirs of infection to other dogs and humans since they are unlikely to receive any prophylactic anthelmintic drugs because of their seemingly healthy status.

The significance of leukocytosis and eosinophilia in dogs with GI parasitism has been documented in Nigeria (Ogunkoya *et al.*, 2006) and India (Qadir *et al.*, 2010). However, this is the first time that this is being documented in Zambia. In agreement with Ogunkoya *et al.* (2006), this study demonstrated a statistically

significant ( $p < 0.05$ ) eosinophilia in dogs with *T. leonina* compared to those that were uninfected. However, except for *T. vulpis* ( $P < 0.05$ ), we did not observe statistical difference in the mean eosinophil levels between dogs parasitized with other GI helminths and non-parasitized ones. The finding of a low differential eosinophil count in dogs parasitized with *T. vulpis* compared to those without the parasite was unusual. However, no possible explanation could be attributed to this finding. A tendency toward an increase in the mean eosinophil percent was observed in dogs with two and three GI helminths compared to the non-parasitized ones. A significant increase in eosinophils is a recognized sign of parasitic infection (Qadir *et al.*, 2010). Parasites that produce an increase in eosinophils are those that penetrate the tissues of the animal body such as migrating ascarid larvae and occasionally hookworms whereas those that produce only localized lesions (e.g. *D. caninum* and Taeniid eggs) do not usually induce an eosinophilia (Embert, 1986). This is consistent with our observations in which *T. canis* indicated a tendency towards an increase in eosinophils in parasitized dogs.

### **5.3 Prevalence of helminth parasites from postmortem studies**

The prevalence of helminth parasites recovered from euthanized dogs in the present study were very high (100 percent) perhaps because many of the dogs in the study were stray dogs that were not better cared for and not restricted than those in other studies. This was higher than the findings in Mexico by Canto *et al.* (2010). It is particularly noteworthy that from this study, in agreement with the findings by Canto *et al.* (2010), *A. caninum* was the most prevalent zoonotic GI helminth followed by *D. caninum* contrary to the findings by Islam and Chizyuka (1983) who found a higher

prevalence of *D. caninum*. This difference could be attributed to the fact that many dogs in the study by Islam and Chizyuka (1983) were owned, possibly better cared for and more restricted with limited access to natural prey, to various small mammals, in addition to raw meat and offals from domestic and wild ungulates than those in our study. The finding of a higher prevalence of *A. caninum* in euthanized dogs is comparable with faecal flotation findings in this study.

Interestingly, multiple infections involving nematodes and cestodes were more prevalent than single infections compared to the results obtained from the faecal flotation methods, which indicated a higher prevalence of single infections with nematodes. This is because in most cases, tapeworms are missed on faecal examination and the fact that the number of eggs released in a given faecal sample can be variable, sometimes there are not any even though the dog has the parasite in its intestines (Ravel, 1995).

Furthermore, the results of this study differ from the findings by Canto *et al.* (2010) who found similar prevalences of single and multiple infections. The findings of our study highlights the complete absence of veterinary care for stray dogs or to the fact that a high percentage of these mixed infections were produced by the combined presence of *A. caninum* and *D. caninum* and this is in agreement with Canto *et al.* (2010). In Nigeria, selective and targeted control programmes have been adopted where 25 percent of stray dogs' population are isolated and treated at least every six months for several years and this has been observed to reduce the morbidity of GI helminths by over 70 percent (Okoye *et al.*, 2010). This approach has been observed to

be cost effective and has been strongly recommended for control programmes in other tropical areas currently experiencing an upsurge in zoonotic infections.

The prevalence of *S. lupi* was higher than previously reported in Zambia (Islam and Chizyuka, 1983), South Africa (Minnaar *et al.*, 2002), Congo DR (Schandevyl *et al.*, 1987) and Mexico (Canto *et al.*, 2010). However, the prevalence of *T. canis* was lower than previously reported. No reason could be advanced as to why the prevalence of *S. lupi* was higher than previously reported in Zambia, South Africa and Mexico. However, in Congo DR we can speculate that the prevalence of *S. lupi* was lower than the finding in this study because faecal examination was the only diagnostic technique used in Congo DR compared to our situation where postmortem examination was carried out. Furthermore, faecal examination for *S. lupi* has limitations in that the shedding of eggs is often intermittent hence repeated faecal samples are sometimes required when the first examination is negative (Dvir *et al.*, 2010).

There was no positive case of *T. spiralis* reported from the postmortem study in Lusaka district using trichinelloscopy. Two cycles of *Trichinella* infections namely domestic and sylvatic cycles have been described (Pozio, 2001). The term “domestic cycle” refers to the transmission pattern occurring in a swine herd for the following reasons: the consumption of uncooked pork scraps from dining rooms, kitchens, restaurants, and slaughterhouses; the consumption of garbage (i.e., garbage-fed pigs); direct pig to pig transmission due to tail or ear bites or to eating swine carcasses that are not promptly removed from the herd; and transmission through synanthropic animals living near the swine herd (e.g., rats, mustelides, and foxes). The sylvatic cycle is that which occurs in nature among wild carnivores with cannibalistic and

scavenger behavior. This cycle occurs virtually throughout the world. However, epidemiological surveys have been carried out only sporadically and there is no information on the sylvatic cycle in many countries including Zambia. We can therefore speculate that the obtained results in domestic dogs in Lusaka could be because most dogs often have no access to woods where they are expected to be infected by consumption of infected intermediate hosts. Furthermore, dogs in Zambia are unlikely to be fed meat from wildlife and pork sold on the market has passed meat inspection, and is not likely fed to dogs either. The results of this study should however be taken with caution because the diagnostic technique used in our study based on trichinelloscopy has been found not to be a very sensitive method in the detection of low to moderate infections (Beck *et al.*, 2005). Therefore, in line with the shortcomings of trichinelloscopy in the detection of *T. spiralis* more sensitive diagnostic techniques like the artificial digestion method should have been undertaken as observed by Beck *et al.* (2005). In addition, the sample size of euthanized stray dogs in this study was limited and restricted only to Lusaka, an urban area and thus there is need to extend the study population to include dogs in rural areas.

Anaemia is the principal consequence of *A. caninum* infection and is related to the blood loss in the intestines, which is associated with the feeding habits of the adult parasites (Qadir *et al.*, 2010). Further, the severity of the clinical disease is related to the intensity of infection, age, nutritional status, iron reserves and presence of acquired immunity. The correlation coefficients in this study had indicated a negative correlation between *A. caninum* burden and PCV. However, this correlation was not statistically significant in the reduction of PCV ( $p > 0.05$ ). We can then conclude from

this finding that intensity of *A. caninum* alone ranging from zero to 223 worms per dog, in those aged above three months with presumably acquired immunity and with enough iron reserves cannot cause significant reduction in the PCV. Severely affected dogs by *A. caninum* are puppies, which have acquired substantial burdens of the worms by the lactogenic route (Lefkaditis, 2001). Puppies of the smaller breeds also suffer relatively more severely than those of larger breeds, but in all new born puppies iron reserves are marginal and milk is a poor source of iron. Older puppies and dogs i.e. those that were mostly sampled in this study, tend to have adequate iron reserves and there is rapid erythropoietic response which compensates for the blood loss (Hoskins, 2001; Lefkaditis, 2001). It would be desirable to carry out controlled experimental single infection with *A. caninum* that will address all the other factors (e.g. nutritional level, presence/absence of acquired immunity and levels of iron reserves) in order to establish infection intensity of *A. caninum* required to cause various degrees of anaemia in Zambian dogs.

## CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study has determined the prevalence of canine endoparasites in Zambia and demonstrated a higher presence of GI helminths than previously reported by Islam and Chizyuka (1983) of which some have public health significance. On the other hand, a low prevalence of canine babesiosis was recorded in agreement with recent reports by Nalubamba *et al.* (2011) with no positive cases of canine trypanosomosis. Of the zoonotic GI helminths *A. caninum* was the most prevalent parasite in both districts with *T. canis* and *D. caninum* being the other zoonotic helminths reported in this study.

The prevalence of all the six reported GI helminths in both dog populations was determined with significant difference being observed only for *D. caninum* and *T. vulpis* with both parasites being higher in Katete than Lusaka. Furthermore, although additional species may be associated with intestinal parasitism in dogs, outbreaks of disease in Lusaka and Katete are most likely to be associated with the six species reported in each district.

Based on the findings of the study, we can also conclude that eosinophilia, reduction in PCV and TPP are not common features associated with intestinal helminths parasitism in clinically normal dogs in Zambia. However, cases of canine babesiosis tend to have a significant reduction in the PCV of infected dogs.

The results also indicate the need for increased veterinary services both in rural and urban areas for the purpose of surveillance programs and routine preventive health measures such as vaccination and parasite control and for educating pet owners on the importance of regular ectoparasite and helminth control and monitoring as a way of reducing the risk that these pets may pose to owners and the public. It will also be desirable to do future research on molecular studies of canine babesiosis and trypanosomosis on the archived samples to determine molecular prevalence of the two parasites in Zambia and identify them to species or subspecies level.

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## CHAPTER 8 ANNEXES

### Annex 8.1 - Distribution of the breeds of dogs in Lusaka and Katete

Breed	Lusaka ( <i>n</i> = 326)		Katete ( <i>n</i> = 160)		Overall ( <i>n</i> = 486)	
	n	Frequency (%)	n	Frequency (%)	n	Frequency (%)
Mongrel	279	86	160	100	439	90
GSD	22	6.7	0	0	9	3.2
Rottweiler	4	1.2	0	0	3	0.9
Boxer	4	1.2	0	0	4	1.2
Boerboel	3	0.9	0	0	3	0.9
Jack Russell	4	1.2	0	0	4	1.2
Fox terrier	1	0.3	0	0	1	0.3
Poodle	4	1.2	0	0	2	0.6
Doberman	2	0.6	0	0	2	0.6
Miniature Schnauzer	1	0.3	0	0	1	0.3
Great Dane	2	0.6	0	0	2	0.6

n = sample size of dogs

Annex 8.2 - Distribution of the age of dogs in months in Lusaka and Katete

Age	Lusaka ( <i>n</i> = 325)		Katete ( <i>n</i> = 160)		Overall ( <i>n</i> = 485)	
	n	Frequency (%)	n	Frequency (%)	n	Frequency (%)
>3months	8	2.5	11	6.9	19	3.9
3-6months	41	12.6	33	20.6	74	15.2
>6-12months	52	16	27	16.9	79	16.3
>12-24months	97	29.8	43	26.9	140	28.9
>24-60months	85	26.2	41	25.6	126	26
>60months	42	12.9	5	3.1	47	9.7

Annex 8.3 - Katete mean %PCV and total plasma protein and number of GI helminth co-infections

	No. of co- infections	N	Mean	Std. Deviat.	95% Confidence Interval for Mean			
					Lower bound	Upper bound	Minimum	Maximum
PCV	0	28	46.2	11.1	41.9	50.5	18	59
	1	86	42.6	9.0	40.6	44.5	16	60
	2	36	39.1	8.5	36.2	42.0	15	56
	3	9	39.9	6.9	34.6	45.2	31	55
	Total	159	42.3	9.4	40.8	43.7	15	60
P_PROTEIN	0	28	7.9	1.2	7.	8.4	5.0	10.0
	1	86	8.2	5.2	7.1	9.3	4.0	54.0
	2	36	7.4	1.3	7.0	7.8	5.0	9.8
	3	9	7.6	1.2	6.7	8.5	6.0	10.0

	No. of co-	N	Mean	Std. Deviat.	95% Confidence Interval for Mean			
					Lower bound	Upper bound	Minimum	Maximum
PCV	0	28	46.2	11.1	41.9	50.5	18	59
	1	86	42.6	9.0	40.6	44.5	16	60
	2	36	39.1	8.5	36.2	42.0	15	56
	3	9	39.9	6.9	34.6	45.2	31	55
	Total	159	42.3	9.4	40.8	43.7	15	60
P_PROTEIN	0	28	7.9	1.2	7.	8.4	5.0	10.0
	1	86	8.2	5.2	7.1	9.3	4.0	54.0
	2	36	7.4	1.3	7.0	7.8	5.0	9.8
	3	9	7.6	1.2	6.7	8.5	6.0	10.0
	Total	159	7.9	3.9	7.3	8.5	4.0	54.0

Annex 8.4 - Lusaka mean %PCV and total plasma protein and number of GI helminth co-infections

	No. of co- infections	n	Mean	Std. Deviat.	95% Confidence Interval for Mean			
					Lower bound	Upper bound	Minimum	Maximum
PCV	0	58	38.2	9.8	35.7	40.8	12.0	60.0
	1	157	38.7	8.1	37.4	40.0	9.0	61.0
	2	32	41.4	8.0	38.5	44.3	22.0	55.0
	3	2	32.5	2.1	13.4	51.6	31.0	34.0
	Total	249	38.9	8.6	37.8	39.9	9.0	61.0
P_PROTEIN	0	58	7.7	1.8	7.3	8.2	3.0	12.0
	1	157	7.7	1.1	7.5	7.8	5.2	10.6
	2	32	7.7	.97	7.4	8.1	6.0	10.2
	3	2	8.0	.85	.38	15.6	7.4	8.6
	Total	249	7.7	1.3	7.5	7.9	3.0	12.0

Annex 8.5 – Overall mean %PCV and total plasma protein and number of GI helminth co-infections

	No. of co-infections	n	Mean	Std. Deviation	95% Confidence Interval for Mean			
					Lower bound	Upper bound	Minimum	Maximum
					PCV	0	86	40.8
	1	243	40.1	8.6	39.0	41.1	9.0	61.0
	2	68	40.2	8.3	38.2	42.2	15.0	56.0
	3	11	38.5	6.9	34.0	43.2	31.0	55.0
	Total	408	40.1	9.1	39.3	41.0	9.0	61.0
P_PROTEIN	0	86	7.8	1.6	7.4	8.1	3.0	12.0
	1	243	7.8	3.2	7.4	8.3	4.0	54.0
	2	68	7.6	1.1	7.3	7.8	5.0	10.2
	3	11	7.7	1.1	6.9	8.4	6.0	10.0
	Total	408	7.8	2.7	7.5	8.0	3.0	54.0

Annex 8.6- Data entry protocol for canine endoparasites

Date: \_\_\_\_\_ Sample ID Number: ( \_\_\_\_\_ ) to be inscribed on all related samples  
 Breed: Mongrel / GSD - X / Rottweiler - X / Poodle - X / Other (specify) \_\_\_\_\_  
 Sex: Male. / Female / Intact / Neutered \_\_\_\_\_ Body Wt.: \_\_\_\_\_  
 Age\* : < 3mo / 3mo-6mo / 6mo – 12mo / >1 - 2 yrs / 2 - 5 yrs / >5 -10yrs / >10yrs  
 Description: \_\_\_\_\_ Residential Area: \_\_\_\_\_  
 Euthanized/Alive \_\_\_\_\_ Last Deworming: \_\_\_\_\_  
 Temperature: \_\_\_\_\_ Body condition score\*\*:  
 CRT: \_\_\_\_\_ MM:  
 Ectoparasites \*: None./ear ticks / body ticks / fleas / lice  
 (Species: \_\_\_\_\_ )  
 Lymphadenopathy \*: Yes / No Lnn affected\* : Submand' / Prescap' / Poplit' /other:  
Circle the appropriate\*\* On a Scale of 1 – 5 (1 = poor; 3=Average/Fair; 5 =obese

PCV	TPP

LYMPH	NEUTR BAND	NEUT SEGM	EOSIN	BASOPH	MONOC

Parasites	Haematology examin. results	Faecal examination results	No. of Worms recovered on PM	Faecal culture results
<i>T. brucei</i>				
<i>Microfilaria</i> species				
°Other blood parasites				
<i>A. caninum</i>				
<i>Trichinella</i> species				
<i>Trichuris</i> species				
<i>T. canis</i>				
<i>C. aerophila</i>				
<i>D. caninum</i>				
<i>E. granulosus</i>				
<i>T. multiceps</i>				
Taeniid eggs				
<i>Cryptosporidium</i> species				
<i>G. lamblia</i>				
Other parasites				

Annex 8.7- Number of worms recovered per euthanized dog as stratified by age and sex

Dog No.	Age category	Sex	<i>A. caninum</i>	<i>T. canis</i>	<i>D. caninum</i>	<i>S. lupi</i>
1	>12mo-24mo	M	10	0	+ve	+ve
2	>12mo-24mo	M	50	0	+ve	-ve
3	>6mo-12mo	F	10	0	-ve	-ve
4	>60mo	M	40	0	+ve	-ve
5	>24mo-60mo	F	20	0	+ve	+ve
6	>24mo-60mo	M	210	0	+ve	+ve
7	>3mo-6mo	F	155	0	+ve	+ve
8	>12mo-24mo	F	115	0	-ve	-ve
9	>3mo-6mo	M	10	0	-ve	-ve
10	>24mo-60mo	M	10	0	+ve	-ve
11	>24mo-60mo	F	15	0	+ve	-ve
12	>24mo-60mo	F	5	0	-ve	-ve
13	>6mo-12mo	F	15	0	-ve	-ve
14	>12mo-24mo	M	40	0	-ve	-ve
15	>24mo-60mo	F	30	0	-ve	-ve
16	>24mo-60mo	M	85	0	+ve	+ve
17	>6mo-12mo	F	75	0	+ve	+ve
18	>24mo-60mo	M	0	0	+ve	-ve

## Annex 8.7 continued

<b>Dog No.</b>	<b>Age category</b>	<b>Sex</b>	<b><i>A. caninum</i></b>	<b><i>T. canis</i></b>	<b><i>D. caninum</i></b>	<b><i>S. lupi</i></b>
19	>60mo	M	0	10	+ve	+ve
20	>12mo-24mo	F	25	0	-ve	-ve
21	>12mo-24mo	M	30	0	-ve	-ve
22	>12mo-24mo	M	5	0	-ve	-ve
23	>60mo	M	1	0	+ve	-ve
24	>24mo-60mo	F	26	0	+ve	-ve
25	>12mo-24mo	M	60	0	+ve	+ve
26	>24mo-60mo	F	34	0	-ve	-ve
27	>24mo-60mo	F	5	0	+ve	-ve
28	>24mo-60mo	M	223	0	+ve	+ve
29	>6mo-12mo	M	115	0	-ve	-ve
30	>24mo-60mo	M	16	0	+ve	-ve
31	>12mo-24mo	F	4	0	+ve	-ve
32	>24mo-60mo	F	4	1	+ve	-ve
33	>24mo-60mo	M	24	0	+ve	-ve

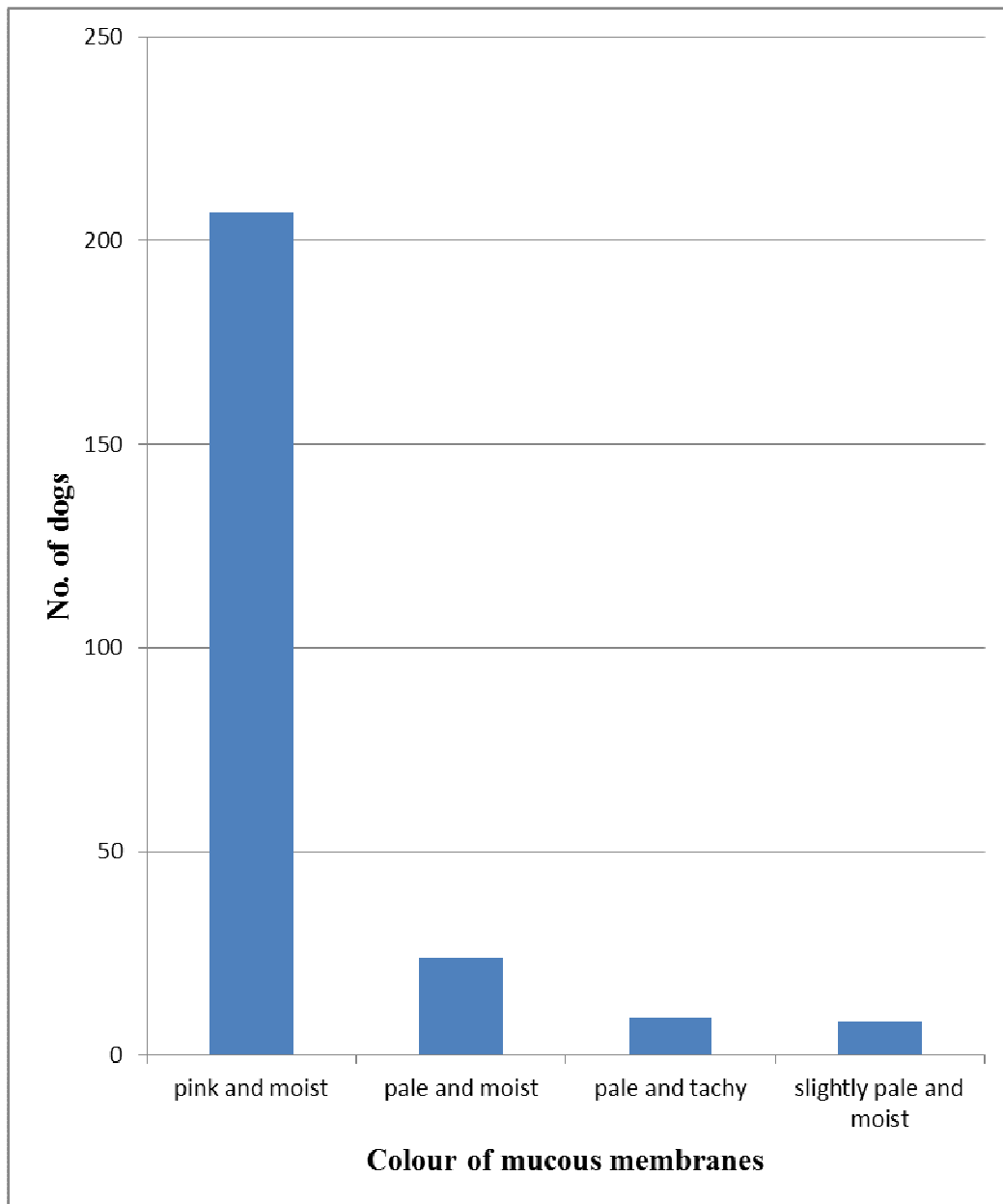
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M = male, F = female

Annex 8.9- Number of dogs with lymphadenopathy as stratified by the lymph node (s) affected

<b>Lymph node (s) affected</b>	<b>No. of dogs</b>	<b>Frequency (%)</b>
No lymph affected	211	44.6
Submandibular affected	58	12.3
Prescapular affected	42	8.9
Popliteal affected	37	7.8
All 3 affected	63	13.3
Submandibular & prescapular affected	20	4.2
Submandibular & popliteal affected	23	4.9
Prescapular and popliteal affected	19	4.0
Total	473	

Annex 9.0- Number of dogs with the colouration of mucous membranes



Annex 9.1: The overall mean differential leukocyte count of dogs.

	<b>Segmented neutrophils</b>	<b>Band neutrophils</b>	<b>Lymphocytes%</b>	<b>Eosinophils %</b>	<b>Monocytes %</b>
	<i>%</i>	<i>%</i>			
n	310	310	310	310	310
Mean	58.7	1.9	26.6	7.8	4.9
±SD	12.7	2.1	11.3	4.8	2.8
Range	72	18	63	26.0	21
Minimum	14	0	4	.0	0
Maximum	86	18	67	26.0	21