PREVALENCE OF *DIROFILARIA IMMITIS* IN DOGS IN ZARIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN EDUCATIONAL BIOLOGY

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NIGERIA

MARCH, 2015

DECLARATION

I hereby declare that the work in this thesis titled **'PREVALENCE OF *DIROFILARIA IMMITIS* IN DOGS IN ZARIA'** was performed by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree at any institution. I take exclusive responsibility for all errors and oversights in this project.

Harrison Ojonimi ABAH _____ _____

Signature Date

CERTIFICATION

This thesis titled **'PREVALENCE OF *DIROFILARIA IMMITIS* IN DOGS IN ZARIA'** by **Harrison Ojonimi ABAH** meets the regulations governing the award of the degree of Master of Science (M.Sc.) of the Ahmadu Bello University, Zaria and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

This project work is dedicated to Almighty God for his guidance and protection throughout my academic pursuit in spite of all odds

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I give the Almighty God all the glory and honour for his knowledge and wisdom. I am also grateful to Him for giving me the faith and abundant grace to start and finish this work. Let His name alone be praised forever, Amen.

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ABSTRACT

The study on prevalence of *Dirofilaria immitis* was conducted in Zaria. A total of three hundred and seventeen dogs were examined from seven sampling sites; Samaru, Sabon Gari, Tudun Wada, Wusasa, Basawa, Kongo and Gyellesu from April to December 2012. 5mls of blood from the branchiocephalic vein was aseptically collected from each dog and was stored in sample bottle containing ethylene diamine tetra acetic acid (EDTA, 1mg/ml) and were transported to the laboratory in a cold box and analyze immediately on arrival, using modified knotts method. The overall prevalence of confirmed cases of *Dirofilaria immitis* infection in the dog population in Zaria was (48) 15.1%. Age specific prevalence showed that, infection was detected throughout the age 1 – 2 years old to 9 years above. Although the 1 – 2 years showed a high rate of infection in 66 dogs (21.2%) than the other ages respectively. The male dogs were relatively more infected than the females, 40 (81.9%) and 8 (8.3%) respectively. The disease was detected in all the sites. The foreign breeds were found to be free from the infection, these is probably due to the fact that special care are given to them. Prevalence was higher in un-caged and caged dogs. 34 (15.2%) and 14 (14.9%) respectively. There was not significant difference ($p>0.05$) in prevalence. A higher percentage of the positive cases showed low haemoglobin level 8 (21.6%), The reason could be that the infection causes anemia which in turn affects the level of hemoglobin likewise the total protein count 16 (13.2%). WBC count increased in positive cases which is indicative of Dirofilaria infection while the RBC is low in positive cases 7(14.8%).These indicates that the presence of the parasite does not affect the RBC. It not significant ($p > 0.05$) in blood counts.

Differential count of the WBC showed that the presence of the infection led to the release of a greater number of white blood cells such as Eosinophils, Lymphocytes, Monocytes and Neutrophils.

Analysis of the blood parameters showed positive correlation between the packed cell volume and haemoglobin, packed cell volume and red blood cell, haemoglobin and red blood cells, while the Neutrophil and lymphocytes showed negative correlation. The result of the study shows that *Dirofilaria immitis* infection is prevalent among dogs in Zaria, and indicates that *D. immitis* infection may be of public health concern then wither to envisaged .

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CHAPTER ONE

1.0 INTRODUCTON

1.1 Background of Study

Dirofilaria is a nematode parasite that is widely enzootic in carnivores especially dog. It is of the family filariidae (Soulsby, 1982). There are two known species of importance in dogs: *Dirofilaria repens (D. repens)* and *Dirofilaria immitis (D. immitis)*, of which *D. immitis* is more important and is commonly called the dog heart worm. The adult worms are found in the right ventricle and pulmonary arteries of dogs and mammals (Gerald and Larry, 1989; Urguhart *et al.,* 2003) and are responsible for delabitating condition known as canine heart worm (CHW) disease or dirofilarosis. Dirofilarosis cause by *D. immitis* is zoonotic and is transmitted by the mosquito vector (Urguhar *et al.,* 2003)**.**

Heartworms go through several live stages before they become adults to infect the pulmonary artery of the host animal. The worms require the mosquito as an intermediate host in order to complete their life cycle. The rate of development in the mosquito is temperature dependent, requiring approximately two weeks of temperature at above 27^{o}C (80^{o}F). Below a threshold temperature of 14^{o}C, development cannot occur, and the cycle will be halted (Knight, 2000). As a result, transmission is limited to warm months and duration of the transmission season varies geographically. The period between the initial infection when the dog is bitten by a mosquito and the maturation of the worms into adults living in the heart takes six(6) to seven(7) months and its known as the "prepatent period".

Clinically, the signs of *D. immitis* infection in dogs are laziness, exercise intolerance, and chronic soft cough with haemophisis. In later stage there are dyspnoe, sometimes edema of the lower limbs and escites, haemoglobnuria, icterus, and collapse of the host usually due to venacaual syndrome (Urguhart *et al.*, 2003).

Canine heartworm infection can be diagnosed based on the clinical signs of cardiovascular dysfunction; demonstration of microfilaria in the blood; thoracic radiography showing thickening pulmonary artery and/ or a positive enzyme linked immunosurbent Assay (E.L.I.S.A) immunochromatography test system. At postmortem, presence of worms in the right ventricle and pulmonary artery are diagnostic.

Dirofilariasis manifests either as subcutaneous nodules or asymptomatic parenchyma disease in human. These lesions are often misdiagnosed as malignant tumors, requiring invasive investigation and surgery (Bionote, 2010).

Heartworm has now spread to nearly all locations where the mosquito vector is found. Transmission of the parasite occurs in all of the United States (cases has been reported in Alaska and the warmer regions of Canada). The highest infection rates are found within 150 miles of the coast from Texas to New Jersey, and along the Mississippi river and its major tributaries. It has also been found in South America, Southern Europe, Southern Asia, The Middle East, Australia, Korea and Japan. (Edward, 2003).

In Nigeria, (Oduye *et al*, 2002) stated that heartworms *(D. immitis)* have been reported in southern Nigeria but not in the northern Nigeria. However, Anyanwu *et al.* (1996), reported the isolation of *D. immitis*-like microfilaria in four Nigerian dogs in Zaria. The four cases were mixed infection with *D. repens*. In a later study, he reported the

abundance of mosquito in the study area namely; culex papeins spp. Aedes aegypti and *Aedes vitatus* (Anyanwu, 2000). Virtually, all these species of mosquitoes are potential vector of *D. immitis*.

In recent past, parasites invasion have been on the increase and constitute a threat to public health. The increasing association between man and dogs and the recent general insecurity in the country has necessitated the importation of exotic breeds of dogs from Dirofilariasis endemic area particularly to the homes of wealthy Nigerians(Benjamin Edward, 2003).

The dog heartworm is of negligible public health risk, because it is unusual for humans to become infected. In addition, human infections are usually of little or no consequences, although rarely, an infected human may show signs of respiratory disease. In most cases, however the heartworm dies shortly after arriving in the human lungs and a nodule known as a granuloma, forms around the dead worm as it is being killed and absorbed. This may well be the most significant medical consequence of human infection by the dog heartworm (Benjamin and Edward, 2003).

The goal of this study is to estimate the prevalence status of *Dirofilaria immitis* in dogs in Zaria, Kaduna state, Nigeria.

Apart from the work done in Southern Nigeria by Oduye and Dipeolu, 2002 and the reported cases of abundances of mosquitoes in Zaria by Anyanwu, 2000 to the best of our knowledge, there is no report on the prevalence of *Dirofilaria immitis* in Zaria, Kaduna State. As reported by WHO (1995), such a comprehensive study is necessary because it is important to know whether an organism causing disease in a given area is of the biotype as in the local sand fly vectors or in putative animal reservoirs. Furthermore,

In developed countries, *Dirofilaria immitis* infection has long been recognized as a health hazard to dogs and cats and is been controlled vigorously because of its zoonotic significance to human (McCall *et al.,* 2004). According to Idowu (2002), control strategies require information on prevalence, incidence, and health status of the disease. These facts explained the need to undertake this research.

1.2 Statement of the Research Proble

Since *D. immitis* was first reported in the South by Oduye and Dipeolu (1976) and the report of suspected cases by Anyanwu *et al.* (1996; 2000). The disease prevalence especially in dogs has remained poorly researched and reported.

Therefore, the extent of the significance of the disease remain unknown in many parts of Nigeria including Zaria.

1.3 Justification

The *Dirofilaria immitis* of dogs are of public health importance, as their burden can cause much morbidity not only to dogs, but also to human. The finding of this research will therefore, provide useful data to the pool of information needed for effective intervention programmes and subsequent control of the *Dirofilaria immitis infection* in Zaria and Nigeria generally.

It is hoped that the results of this study will further provide reliable data for the determination of the prevalence, and public health significance of the disease towards meaningful planning of appropriate control measures against the disease in the study area and beyond.

1.4 Aim

The aim of this study is to evaluate the prevalence of *Dirofilaria immitis* infection in dogs in Zaria, Kaduna State.

1.5 Objectives

The major objectives of the study are to:

1. determine the prevalence of *Dirofilaria immitis* (heartworm) in dogs in Zaria, Kaduna State.
2. Determine D. Immitis infection in relation to the sex, age and breed in dogs in Zaria.
3. determine the factors that predisposing dogs to *Dirofilaria immitis in Zaria.*

1.6 Hypotheses

1. There is no heartworm infection in dogs in Zaria.
2. D.immitis infection is not significantly influenced by the sex, age and the breed of dogs in Zaria.
3. There are no risk factors to heartworm disease in Zaria.

1.7 Limitation of the study

The variation in the sample sizes across the various sites in Zaria was caused by a number of unforeseen circumstances. Such factors include differences in population sizes of dogs in the sites, denial of access to selected available dogs by dog owners, absence of dogs owners at home at the time of visit, absence of dogs at home at the time of visitation, some dogs are violent, even to the dog owners and to the point that they have to be left aside and lack of time, finance and research logistic problems

The problems could possibly introduce some bias in the sampling but Theis (2005) attributed such bias to non-compensating errors because increasing the size of the sample cannot reduce them.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 Morphology of *Dirofilaria immitis*

Dirofilaria immitis is a cylindrical slender white nematode worm. It has a cuticle with three main outer layers made of collagen and other compounds. The outer layers are non cellular and are secreted by the epidemic. The cuticle layer protects the nematodes so they can invade the digestive tracts of animals (Claudio *et al.,* 2001).

Nematodes have longitudinal muscles along the body wall. The muscles are obliquely arranged in bands, dorsal, ventral and longitudinal nerve cords are connected to the main body of the muscle.

Both sexes are different. The adult male, measuring 12 – 16 cm smaller than the adult female which is 25 – 30 cm. The male has a posterior end spirally coiled and a tail with many alae, which are thickenings of the cuticle. The female posterior is straight. Both sexes have a mouth, a filariform esophagus, anal pore, excretory pore and a nerve ring. The male has a seminal vesicle and a testis while the female bears on ovary and oviduct (Claudio *et al.,* 2001).

The larvae, called microfilaira are 307-322 micro meters long and 6.7-7.1 micro meters wide. They have a straight posterior and regardless of the sex and a tapered anterior end. They have no cephalx hook and are not ensheated (Claudio *et al.,* 2001).

Kaewthamasorn (2008) classified *Dirofilaria immitis* as to the kingdom Animalia, phylum Nematoda, class Secernentae, Order Spiruvida, Family Onchocercidae, Genus *Dirofilaria,* Species *Dirofilaria immitis*

2.2 Types of Dirofilaria Worms

Two main Filarial worms parasitizing dogs are;

(a) ***Dirofilaria immitis****:* Is a common parasite of dogs (and some other carnivores) in many parts of the world. It is considerably veterinary problem, particularly in U.S.A and Japan. In the dogs, adult worms lie coiled in tangled masses in the right ventricle of the heart. The microfilaria circulates in the blood and is transmitted by mosquitoes (Clarke, 2005).

(b) ***Dirofilaria repens***: is also a common filarial parasite and dogs serves as the natural host in various regions of the world. *D. repens* has also been documented to be pathogenic in man where it may form coetaneous nodules on various parts of the human body (Anyanwu *et al.,* 2000).

2.3 The Vectors of *Dirofilaria immitis* of Dogs

Some 60 species of mosquitoes have been recorded as vectors of this species. Since many of these attack man, it is not surprising to find that man is occasionally infected. Some 100 human cases have been reported in literature, and the predilection sites being the heart and lung tissues (Anyanwu *et al.,* 2000).

2.4 Life Cycle of *Dirofilaria immitis*

Heartworms go through several life stages before they become adults infecting the pulmonary artery of the host animal. The worms require the mosquito as an intermediate host to complete their life cycles (fig. 2.1). The rate of development in the mosquito is temperature dependent, requiring about two weeks of temperature at or above 27^{o}C (80^{o}F). Below a threshold temperature of 14^{o}C (57^{o}F), development cannot occur and the

cycle will be halted (Knight, 2000). As a result, transmission is limited to warm months, and duration of the transmission season varies geographically. The period between the initial infection when the dog is bitten by a mosquito and the maturation of the worms into adults living in the heart takes period of six to seven months in the dog and is known as the “prepatent period”.

After infection, the third stage of larval heartworm (L3) deposited by the mosquito grow for a week or two and molt to the fourth larval stage (L4) under the skin at the site of the mosquito bite. Then, they migrate to the muscles of the chest and abdomen, and 45 to 60 days after infection, molt to the fifth stage (L5, immature adult). Between 75 and 120 days after infection, these immature heartworms then enter the bloodstream and are carried through the heart to reside in the pulmonary artery. Over the next three to four months, they increase greatly in size. The female adult worm is about 30cm in length, and the male is about 23cm, with a coiled tail (Collin, 1998).

The microfilaria circulates in the bloodstream for as long as two years, waiting for the next stage in their life cycle in the gut of the bloodsucking mosquito. When ingested by a mosquito, the microfilaria undergoes a series of molts to the infective third larval stage, and then migrates to the salivary glands of the mosquito where they wait to infect another host. The incubation period required to reach the stage where the microfilaria becomes transmittable to another host can be as little as two weeks or as long as six weeks, depending on the warmth of the climate, and the larval life cycle ceases entirely if the ambient temperature drops below $14^{o}C$ ($57^{o}F$).

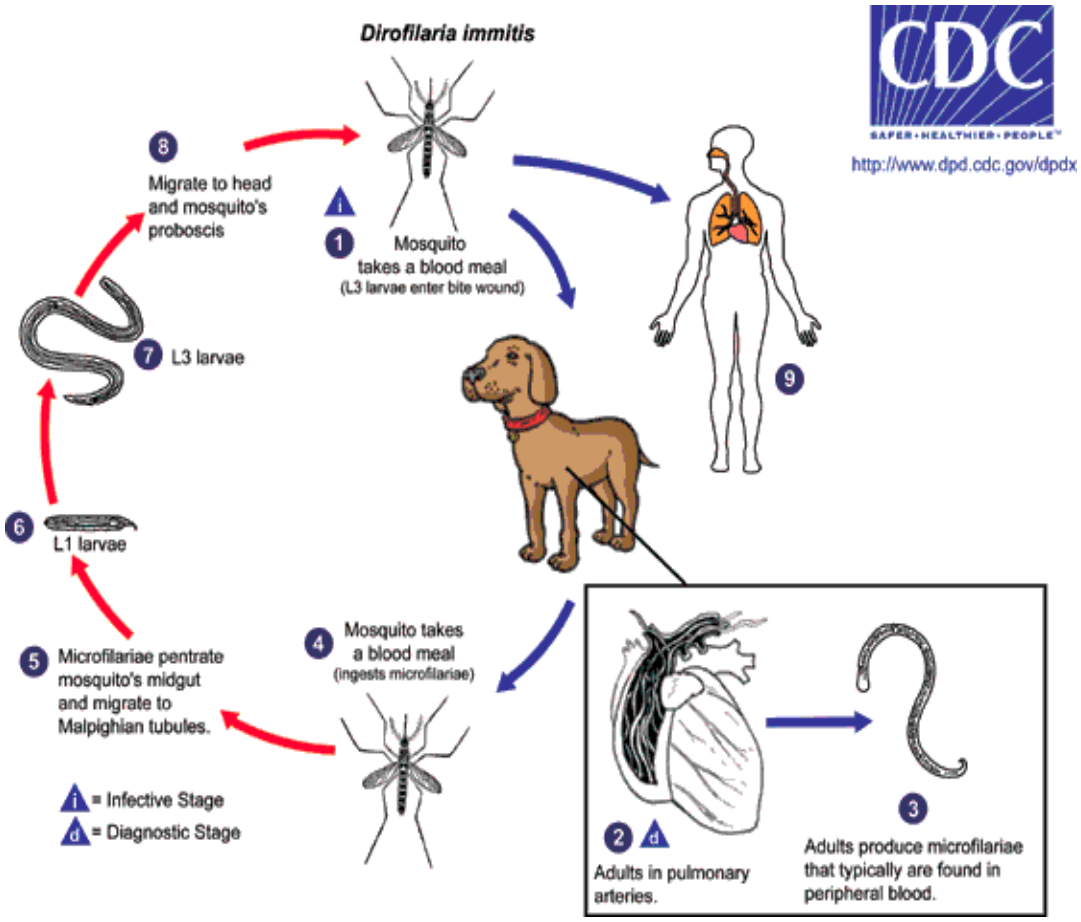

Figure 2.1: Life Cycle of *Dirofilaria immitis*.

2.5 Causes of infection

Dirofilaria repens is a subcutaneous parasite of dogs and cats in Europe, Africa, and Asia and probably accounts for incidental infections in humans in these areas. Both *D. Immitis* and *D. repens* are spread by mosquitoes. Both parasites present a subcutaneous nodule which develop over several weeks and are variously tender, painful, red, and sometimes migratory. They develop in the conjunctiva, the eyelid, scrotum, breast, arms or legs. Rarely, *D. repens* produces pulmonary lesions similar to *D. immitis* (Benjamin and Edward, 2003).

2.6 Clinical Signs of Infection

Dogs show no indication of heartworm infection during the six month prepatent period prior to the worms' maturation, and current diagnosis tests for the presence of *microfilariae* or *antigens* cannot detect prepatent infections. Rarely, migrating heartworms get "lost" and end up in unusual sites, such as the eye, brain, or an artery in the leg, which results in unusual symptoms such as blindness, seizures and lameness. But normally, until the larvae mature and congregate inside the heart, they produce no symptoms or sign of illness.

Many dogs will show little or no signs of infection even after the worms become adults. These animals usually have only a light infection and live a fairly sedentary lifestyle. However, active dogs and those with heavier infections may show the classic signs of heartworm disease. Early signs include a cough, especially on the exercise and early exhaustion upon exercise. In the most advanced cases where many adult worms have

built up in the heart without treatment, signs progress to serve weight loss, fainting, coughing up blood and finally, congestive heart failure (Nassau, 2003).

2.7 Diagnosis

The methods used for the diagnosis include microfilaria detection, antigens testing, immunodiagnostic and x-rays.

2.7.1 Microfilaria detection

Was accomplished most commonly in the past by the microscopic identification of microfilaria on a direct blood smear, above the buffy coat in a microhematocrit tube (or capillary tube), using the modified Knott test, or after Millipore filtration. The accuracy of the tests, typically used for routine screening or diagnosis of heartworm infection, is improved by multiple testing. The modified Knott test and Millipore filtration are more sensitive because they concentrate microfilarae, improving the chance of diagnosis (Edward, 2003). The direct smear technique allows examination of larval motion, helping in the distinction of *Dirofilaria immitis* from Acanthocheilonema reconditum. This distinction is important because the presence of the latter paradise does not require expensive and potentially harmful therapy. However, the potential for a microfilaremic infection is 5-67%. The number of circulating microfilariae does not correlate with the number of adult heartworms, so it's not an indicator of disease severity.

2.7.2 Antigens testing

In most practices, has supplanted or supplemented microfilarial detection. Combining the microfilaria and adult antigen test is most useful in dogs receiving diethylcarbamazine or no preventative (as macrolides as for example ivermectin or moxidectin typically render

the dog a microfilaremic) Up to 1% of infected dogs are microfilaria-positive and antigen-negative.

2.7.3 Immunodiagnostics

(ELISA, lateral flow immunoassay, rapid immunomigration techniques) to detect heartworm antigen in the host's blood are now regularly used. The weakness of these tests is they only detect the antigens released from the adult female worm's reproductive tract, so will produce negative results during the first five to eight months of infection (Benjamin and Edward, 2003). The specificity of these tests is close to 100% and the sensitivity is more than 80% (Nelson *et al.*, 2005). A recent study demonstrated a sensitivity of only 64% for infections of only one female worm, but improved with increasing female worm burden (85%, 88%, and 89% for two, three and four female worms, respectively). Specificity in this study was 97% (Benjamin and Edward, 2003). False-negative test results can be due to low worm counts, immature infections and all male infections.

2.7.4 X-rays

Are used to evaluate the severity of the heartworm infection and develop a prognosis for the animal. Typically, the changes observed are enlargement of the main pulmonary artery, the right side of the heart, and the pulmonary arteries in the lobes of the lung. Inflammation of the lung tissue s also often observed (http://www.heartwormsociety.org/)

2.8 Public Health Implications *Dirofilaria immitis*

Since dogs are domestic animals living with man, dogs filarial parasite i.e the *Dirofilaria immitis* and *Dirofilaria repens* was long considered to be a parasite limited to the tropics

and subtropics, but its distribution is now recognized to be worldwide and is known to be enzootic in Canada and the U.S.A, where high prevalence in dogs, wolves, and foxes have been reported from some areas (Anyanwu *et al.,* 2000) some species of mosquito have been recorded as vectors of this species and since many of these attack man it is not surprising to find that man is occasionally infected. Some 100 human cases being reported in the literature with the heart and lungs being predilection sites (Anyanwu *et al.,* 2000)**.**

Transmission to another host occurs when the infected mosquito takes a blood meal from the dog and infective larvae of filarial after having developed in the infected mosquito, are released from its mouth parts to man, during subsequent blood meal.

2.9 Control of Dirofilariasis

The genus Dirofilariasis, which comprises two important parasites namely; *D. immitis* and *D. repens*. The control depends largely on the elimination of vectors breeding sites and treatment of an infected dog through administration of drugs.

2.9.1 Control measures of dogs filarial infection through vector control

The control of *Dirofilaria immitis* and *Dirofilaria repens,* depends largely on the elimination of mosquito breeding sites.

2.9.2 Control through screening of dogs and therapeutic intervention

Dog screening using laboratory diagnosis and administering vomeic to infected cases has been suggested by Anyanwu *et al.* (2000) A number of other control measures have been suggested by Tarello (2003) which include:

- Many infections are asymptomatic and require screening programms to detect.

- Drugs against the adults or vaccines could be developed.
- Treatment of populations with ivermectin.

2.9.3 Control through health education

Filariae parasitizing both man and animals are of public health significance. The world Health Organization has published a valuable practical manual on all aspect of filariosis control (Tarello, 2003). Control depends largely on the elimination of mosquito breeding sites, which in bancrotian filariosis involves urban mosquitoes, a situation reviewed by Soulsby (1982). Mass chemotherapy has long involved the use of diethyl carbamazine (Tarello, 2003) which can be mixed with salt. Recent results suggested that ivermectin, which is very effective against the microfilariae, can replace D.E.C (Ray and Jack, 2003,). Unfortunately, it does not kill the adult worms and this fact severely limits its value in the eradication of the diseases (Campbell, 1990).

Education programmes carried to all three tiers of our education system on the vectors and their control including the effect of the disease would go a long way in minimizing the effects of the disease of dirofilarosis in man and our pet, the dogs (Harrison and Thompson, 2005).

2.10 Prevention Through Drugs

Prevention of heartworm infection can be obtained through a number of veterinary drugs. The drugs approved for use in the US are ivermectin (sold under the brand names Heartgard, Iverhart and several other generic versions), mlbemycin (interceptor flavor tabs and sentinel flavor tabs) and mixodectin (proHeart) administered as pills or chewable tablets. Mixodectin is also available in both a six-month and twelve-month, sustained

release injections, proHeart 6 or proHeart 12, administered by veterinarians. The injection form of mixodectin was taken off the market in the United States due to safety concerns in 2004, but the FDA returned a newly formulated proHeart 6 to the market in 2008. ProHeart 6 remains on the market in many other countries, including Canada and Japan. Its sister product, proHeart 12, is used extensively in Australia and Asia as a 12 month injectable preventive. Topical treatments are available, as well. Advantage Multi (imidacprid + moxidectin) topical solution, uses moxidectin for control and prevention of round worms, hookworms, heartworms and whipworms, as well as imidacprid to kill adult fleas. Selemactin (Revolution) is a topical preventive likewise administered monthly; it also controls fleas, ticks and mites.

Preventive drugs are highly effective, and when regularly administered, will protect more than 99% of dogs and cats from heartworm. Most compromises in protection result from failure to properly administer the drugs during seasonal transmission periods. In regions where the temperature is consistently above $14^{o}C$ year round, a continuous prevention schedule s recommended.

Lapses of up to four months between doses of ivermectin-based products, still provides 95% protection from adult worms. This period is called the reach-back effect. Annual heartworm testing is highly recommended for pet owners who choose to use minimal dosage schedules.(Asimeng, 1985).

Heartworm prevention for dogs is available as ivermectin (Heartguard for dogs), milbemycin (interceptor), or the topical selamectin (Revolution for dogs) and advantage Multi (imidacprid + moxidectin) topical solution.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Zaria, Kaduna State Nigeria which falls within the Guinea-Savannah Zone and covers an area of about 300km^2 (Figure 3.1). It lies between Longitude 11^{O}E and 7^{O}N. The study was conducted in different study sites in Zaria. The sites include Samaru, Wusasa, Sabon Gari, Tudunwada, Depot/NMS, Basawa and Gyallesu area.

3.2 Study Population

The random sampling technique (Jan and Stephen, 2001) was used. This is based on sampling the most accessible unit of the population. Accessible household dogs and dogs brought to the Veterinary Clinic, Ahmadu Bello University, Zaria were sampled. Three hundred and fifteen dogs were sampled between April and December, 2012. In addition, a questionnaire which captures the bio-data of the dogs was given to the owners to fill.

3.3 Sample Size Determination

Sample size was calculated using the formula;

$N = \frac{Z^2pq}{L^2}$ Where N = sample size,

Z = standard normal distribution at 95% confidence (1.96)

` p = prevalence of 23.3% (Anyanwu, 2000)

q = 1 – p.

N = 272 dogs approximately which is the minimum number of dogs that were sampled.

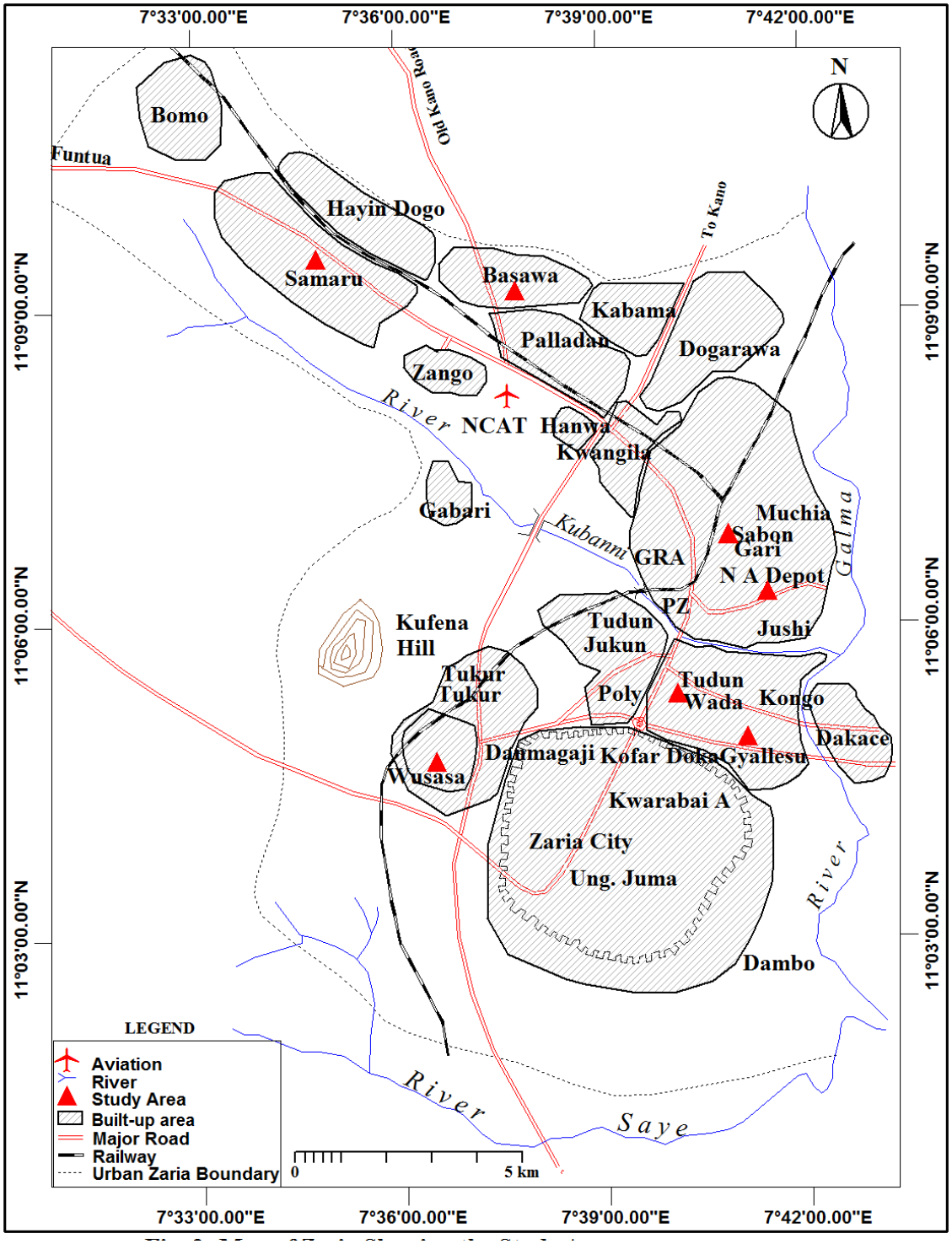

Figure 3.1: Map of Zaria Showing the Study Areas.
Source: Adapted and Modified from Urban Zaria Map, 2013.

The sample population of 317 dogs was obtained from 7 sites. The total sample size per location ranged from 20 – 70 . The variation in the sample sizes across the various sites in Zaria was caused by a number of unforeseen circumstances. Such factors include differences in population sizes of dogs in the sites, denial of access to selected available dogs by dog owners, absence of dogs owners at home at the time of visit, absence of dogs at home at the time of visitation, some dogs are aggressive, even to the dog owners and to the point that they have to be left alone and finance and research logistic problems

The problems could possibly introduce some bias in the sampling but Theis (2005) attributed such bias to non-compensating errors because increasing the size of the sample cannot reduce them.

3.4 Sample collection

About 5mls of blood from the branchiocephalic vein was aseptically collected from each dog using 22 or 21G needle fitted with 10ml syringe. Five milliliters of the blood was stored in sample bottle containing ethylene diamine tetra acetic acid (EDTA, 1mg/ml) for microfilaria examination and complete cell count. Samples were transported to the laboratory in a cold box and analyze immediately on arrival.

3.5 Microfilaria Examination (Modified Knotts Method)

This was done as described by Jay and Marion (1990). A 1ml of blood was placed in EDTA in 15ml centrifuge tube and 10ml of 2% formalin was added and covered with a cover tube mixed by inversion.

After two to three minutes, it was centrifuge for about 5 minutes at 1500rpm and the supernatant was poured off. A drop of 0.1% Giemsa stain was added to the sediment mix

and the pipetted mixture was placed into a clean glass slide, which was covered with a slip and examined under high power microscope (x40) Geisma stained elongated microfilaria was visible in positive samples.

3.5.1 Identification of parasite (*Dirofilaria Immitis*)

Using micrometer eye piece, the cross section of *D. immitis* ranges from 140-300 micrometer in diameter. The cuticle is smooth and lack significant longitudinal ridges. The tail of the microfilaria of *D. immitis* is straight while in repens is curved with button hook. *Dirofilaria immitis* are also motile and numerous when viewed in blood film (Gotta, 2012). Calibrated microscopic length x micrometer eye piece.

3.6 Complete Blood Count (CBC)

Three mililters (3ml) of blood containing EDTA as anticoagulant (1mg/1ml) from each dog was used to determine the following blood parameters. Packed cell Volume (PCV), White blood cell (WBC), Haemoglobin concentration, Red blood cell (RBC), Different count and Total protein. This CBC was analysed using automatology analyser-BC, 2800vet, Mindray Nenshan-Shenzhen 518057, P.R. China.

3.6.1 Procedure for whole blood count using autohematology analyzer

The menu key was pressed to select “Animal” to enter the “Animal” screen, the desired animal was by pressing key. The menu key was pressed to enter the “count” screen. At the “count” screen, the mode key was pressed to select the Whole Blood analysis mode “WB”.

The anticoagulant blood was mixed and placed under the sample probe. The aspirate key was pressed to start the analysis and the results will be displayed on the screen later.

3.7 Data Analyses

The prevalence of the disease was determined by computing the percentage of the number of instances of disease cases (Those with *Dirofilaria immitis* or positive cases) (Sidney, 2004). This was computed as "specific prevalence" and was computed as the percentage of *Dirofilaria immitis* at the total sample population of dogs at risk of the disease at that particular point in time based on age, sex, home of dogs and breed. (Anyanwu, 2000).

The chi-Square analysis (χ^2) was applied to determine whether the differences in prevalence across age groups, sex, site /area, breed and home of dog in the sampled population of Dogs and correlation analysis was used to determine the relationship between blood parameters.

CHAPTER FOUR

4.0 RESULTS

4.1 Age-Specific Prevalence of *Dirofilaria immitis* infection in Dog Population in Zaria

A total of 66 dogs age 1-2 years, 62 dogs age 3-4 years, 66 dogs age 5 – 6 years, 65 dogs age 7 – 8 years and 58 dogs age above 8 years were examined for *D. immitis*. Out of this number, 14 dogs representing 21.2% (1-2 years), 8 dogs representing 12.9% (3-4 years), 10 dogs representing 15.2% (5 -6 years), 8 dogs representing 12.3% (7 – 8 years) and 8 dogs representing 13.8% (8 years and above) were infected. There was no significant difference (P>0.05) between prevalence. (Table 4.1).

4.2 Sex-Specific Prevalence of *Dirofilaria immitis* infection in Dog Population in Zaria

A total of 211 males and 96 females were examined for *D. immitis*. Out of the number, 40 dogs representing 18.1% of males and 8 dogs representing 8.3% of females were infected. There was no significant difference (P>0.05) between prevalence of males and females (Table 4.2)

4.3 Breed-Specific Prevalence of *Dirofilaria immitis* infection in Dog Population in Zaria

A total of 218 local dogs, 85 mixed, ad 14 Alsatian dogs were examined for *D. immitis*. Out of this number, 36 local dogs representing 16.5% and 12 mixed dogs representing 14.1% were infected. No infections were found in the Alsatian dogs. There was no

Table 4.1: Age-Specific Prevalence of *Dirofilaria immitis* Infection in Dog Population in Zaria

Age Group	Number Examined	Number Positive (%)	Chi-square	Df	p value
1 - 2 years	66	14 (21.2)	2.623	4	0.623ns
3 - 4 years	62	8 (12.9)			
5 - 6 years	66	10 (15.2)			
7 - 8 years	65	8 (12.3)			
> 8 years	58	8 (13.8)			
Total	**317**	**48 (15.1)**			

ns – non-significant at P>0.05.

Table 4.2: Sex-Specific Prevalence of *Dirofilaria immitis* Infection in Dog Population in Zaria

Sex	Number Examined	Number Positive (%)	Chi-square	Df	p value	Odd ratio
Male	221	40 (18.1)	0.419	1	0.517	0.81
Female	96	8 (8.3)				
Total	**317**	**48 (15.1)**				

ns – non-significant at P>0.05.

significant difference (P>0.05) between prevalence of local, mixed and Alsatian dogs (Table 4.3).

4.4 Home-Specific Prevalence of *Dirofilaria immitis* infection in Dog Population in Zaria

A total of 94 dogs in the cages and 223 stray dogs were examined for *D. immitis*. Out of this number, 14 caged dogs representing 14.9% ad 34 stray dogs representing 15.2% were infected. There was no significant difference (P>0.05) between prevalence of caged and stray dogs (Table 4.4).

4.5 Site-Specific Prevalence of *Dirofilaria immitis* infection in Dogs Population in Zaria

A total of 67 dogs in Basawa, 24 dogs in Deport/NMS, 47 dogs in Gyellesu, 39 dogs in Sabon Gari, 50 dogs in Samaru, 44 dogs in Tudun Wada and 46 dogs in Wusasa were examined for *D. immitis*. Out of this number, 10 dogs representing 14.9% in Basawa, 4 dogs representing 16.7% in Deport/NMS, 4 dogs representing 8.5% in Gyellesu, 6 dogs representing 15.4% in Sabon Gari, 6 dogs representing 12.0% in Samaru, 8 dogs representing 18.2% in Tudun Wada and 10 dogs representing 21.7% in Wusasa were infected. There was no significant difference (P>0.05) between prevalence of location/site (Table 4.5).

4.6 Analysis of Blood Parameters

4.6.1 *Dirofilaria immitis* in relation to pack cell volume (PCV) of the dogs

A total of 317 dogs were examined for PCV. Out of this number, 122 dogs had low PCV, 148 dogs had normal PCV and 47 dogs had high PCV. Out of this number for

Table 4.3: Breed-Specific Prevalence of *Dirofilaria immitis* Infection in Dog Population in Zaria

Breed	Number Examined	Number Positive (%)	Chi-square	df	p value
Local	218	36 (16.5)	2.887	2	0.236ns
Mixed	85	12 (14.1)			
Alsatian	14	0 (0.00)			
Total	**317**	**48 (15.1)**			

ns – non significant at P>0.05.

Table 4.4: Home-Specific Prevalence of *Dirofilaria immitis* Infection in Dog Population in Zaria

Home	Number Examined	Number Positive (%)	Chi-square	df	p value	odd ratio
Cage	94	14 (14.9)	0.006	1	0.936	0.973
Uncage	223	34 (15.2)				
Total	**317**	**48 (15.1)**				

ns – non-significant at P>0.05.

Table 4.5: Site-Specific Prevalence of *Dirofilaria immitis* Infection in Dog Population in Zaria

Area	Number Examined	Number Positive (%)	Chi-square	df	p value
Samaru	50	6 (12.0)	3.915	6	0.688ns
Wusasa	46	10 (21.7)			
Sabon Gari	39	6 (15.4)			
Depot/NMS	24	4 (16.7)			
Gyllasu	47	4 (8.5)			
Tudun Wada	44	8 (18.2)			
Basawa	67	10 (14.9)			
Total	**317**	**48 (15.1)**			

ns – non-significant at P>0.05.

low, normal and high PCV, 14 dogs representing 11.5% for low PCV, 26 dogs representing 17.6% for normal PCV and 8 dogs representing 17.0% for high PCV were infected. There was no significant difference (P>0.05) between prevalence of low, normal and high PCV (Table 4.6).

4.6.2 *Dirofilaria immitis* in relation to haemoglobin concentration (HC) of the dogs

A total of 317 dogs were examined for HBC. Out of this number, 101 dogs had low HBC, 179 dogs had normal HBC and 37 dogs had high HBC. Out of this numbers for low, normal and high HBC, 3 dogs representing 3.0% for low HBC, 37 dogs representing 20.7% for normal HBC and 8 dogs representing 21.6% for high HBC were infected. There was no significant difference (P>0.05) between prevalence of low, normal and high HBC (Table 4.7).

4.6.3 *Dirofilaria immitis* in relation to total protein count (TPC) of the dogs

A total of 317 dogs were examined for TPC. Out of this number, 73 dogs had low TPC, 123 dogs had normal TPC ans 121 dogs had high TPC. Out of this number for low, normal and high TPC, 1 dog representing 1.4% for low TPC, 31 dogs representing 25.2% for normal TPC and 16 dogs representing 13.2% for high TPC were infected. There was no significant difference (P>0.05) between prevalence of low, normal and high TPC (Table 4.8)

Table 4.6: *Dirofilaria immitis* in Relation to Pack Cell Volume (PCV) of the Dogs

PCV Level	Number Examined	Number Positive (%)	Chi-square	df	p value
Low (<37)	122	14 (11.5)	2.083	2	0.353ns
Normal (37-55)	148	26 (17.6)			
High (>55)	47	8 (17.0)			
Total	**317**	**48 (15.1)**			

ns – non-significant at P>0.05.

Table 4.7: *Dirofilaria immitis* in Relation to Haemoglobin Concentration (HC) of the Dogs

HB level	Number Examined	Number Positive (%)	Chi-square	df	p value
Low (<12)	37	8 (21.6)	17.112	2	0.000**
Normal (12-18)	179	37 (20.7)			
High (>18)	101	3 (3.0)			
Total	**317**	**48 (15.1)**			

** - highly significant at P>0.015

Table 4.8: *Dirofilaria immitis* in Relation to Total Protein Count (TPC) of the Dogs

TPC level	Number Examined	Number Positive (%)	Chi-square	df	p value
Low (<5.4)	73	1 (1.4)	20.813	2	0.000**
Normal (5.4-7.7)	123	31 (25.2)			
High (>7.7)	121	16 (13.2)			
Total	**317**	**48 (15.1)**			

** - highly significant at P>0.05

4.6.4 *Dirofilaria immitis* in relation to white blood cells (WBCs) count of the dogs

A total of 317 dogs were examined for WBC. Out of this number, 50 digs had low WBC, 231 dogs had normal WBC and 36 dogs had high WBC. Out of this number for low, normal and high WBC. 27 dogs representing 54.0% for low, 21 dogs representing 9.1% in normal and 0 dogs representing 0.0% for high WBC were infected. There was high significant difference ($P<0.05$) between prevalence of low, normal and high WBC (Table 4.9)

4.6.5 *Dirofilaria immitis* in relation to red blood cells (RBCs) count of the dogs

A total of 317 dogs were examined for RBC. Out of this number, 54 dogs had low RBC, 215 dogs had normal RBC and 174 dogs had high RBC. Out of this numbers for low, normal and high RBC. 16 dogs representing 13.9% for low, 26 dogs representing 14.9% in normal and 6 dogs representing 21.4% for high RBC were infected. There was significant differences ($P<0.05$) between prevalence of low, normal and high RBC (Table 4.10).

4.6.6 *Dirofilaria immitis* in relation to neutrophil count (NC) of the dogs

A total of 317 dogs were examined for NC. Out of this number, 115 dogs had low NC, 174 dogs had normal NC and 28 dogs had high NC. Out of this numbers for low, normal and high NC, 16 dogs representing 13.9% for low NC, 26 dogs representing 14.9% for normal NC and 6 dogs representing 21.4% for high NC were infected. There was no significant difference ($P>0.05$) between prevalence of low, normal and high NC (Table 4.11).

Table 4.9: *Dirofilaria immitis* in Relation to White Blood Cells (WBCs) of the Dogs

WBC Level	Number Examined	Number Positive (%)	Chi-square	df	p value
Low (<6)	36	0 (0.0)	71.763	2	0.000
Normal (6-17)	231	21 (9.1)			
High (>17)	50	27 (54.0)			
Total	**317**	**48 (15.1)**			

** - highly significant at P<0.05

Table 4.10: *Dirofilaria immitis* in Relation to Red Blood Cells (RBC) of the Dogs

RBC level	Number Examined	Number Positive (%)	Chi-square	df	p value
Low (<14)	48	7 (14.8)	7.014	2	0.030*
Normal (14-20)	215	39 (18.1)			
High (>20)	54	2 (3.7)			
Total	**317**	**48 (15.1)**			

* - significant at P<0.05.

Table 4.11: *Dirofilaria immitis* in Relation to Neutrophil Count (NC) of the Dogs

Neutrophil level	Number Examined	Number Positive (%)	Chi-square	df	p value
Low (<60)	115	16 (13.9)	1.002	2	0.606ns
Normal (60-77))	174	26 (14.9)			
High (>77)	28	6 (21.4)			
Total	**317**	**48 (15.1)**			

ns – non-significant at P>0.05.

4.6.7 *Dirofilaria immitis* in relation to lymphocytes count (LC) of the dogs

A total of 317 dogs were examined for LC. Out of this number, 52 dogs had low LC, 95 dogs had normal LC and 170 dogs had high LC. Out of this numbers for low, normal and high LC, 17 dogs representing 32.7% for low LC, 15 dogs representing 15.8% for normal LC and 16 dogs representing 9.4% for high LC were infected. There were significant differences ($P<0.05$) between prevalence of low, normal and high LC (Table 4.12).

4.6.8 *Dirofilaria immitis* in relation to monocytes count (MC) of the dogs

A total of 317 dogs were examined for MC. Out of this number, 12 dogs had low MC, 268 dogs had normal MC and 37 dogs had high MC. Out of this numbers for low, normal and high MC, 2 dogs representing 16.7% for low MC, 45 dogs representing 16.8% for normal MC and 1 dog representing 2.7% for high MC were infected. There was no significant difference ($P>0.05$) between prevalence of low, normal and high MC (Table 4.13).

4.6.9 *Dirofilaria immitis* in relation to eosinophils count (EC) of the dogs

A total of 317 dogs were examined for EC. Out of this number, 37 dogs had low EC, 274 dogs had normal EC and 6 dogs had high EC. Out of this numbers for low, normal and high EC, 4 dogs representing 10.8% for low EC, 44 dogs representing 16.1% for normal EC and no dog for high EC were infected. There was no significant difference ($P>0.05$) between prevalence of low, normal and high EC (Table 4.14).

Table 4.12: *Dirofilaria immitis* in Relation to Lymphocytes Count (LC) of the Dogs

Lymphocytes level	Number Examined	Number Positive (%)	Chi-square	Df	p value
Low (<12)	170	16 (9.4)	16.84	2	0.000**
Normal (12-30)	95	15 (15.8)			
High (>30)	52	17 (32.7)			
Total	**317**	**48 (15.1)**			

** – highly significant at P<0.05.

Table 4.13: *Dirofilaria immitis* in Relation to Monocytes Count (MC) of the Dogs

Monocytes level	Number Examined	Number Positive (%)	Chi-square	df	p value
Low (<3)	37	1 (2.7)	5.045	2	0.080ns
Normal (3-10)	268	45 (16.8)			
High (>10)	12	2 (16.7)			
Total	**317**	**48 (15.1)**			

ns – non-significant at P>0.05.

Table 4.14: *Dirofilaria immitis* in Relation to Eosinophils Count (EC) of the Dogs

Eosinophils level	Number Examined	Number Positive (%)	Chi-square	Df	p value
Low (<2)	6	0 (0.0)	1.790	2	0.409ns
Normal (2-10)	274	44 (16.1)			
High (>10)	37	4 (10.8)			
Total	**317**	**48 (15.1)**			

ns – non-significant at P>0.05.

4.6.10 *Dirofilaria immitis* in relation to basophils count (BC) of the dogs

A total of 317 dogs were examined for BC. Out of this number, 37 dogs had low BC, 274 dogs had normal BC and 6 dogs had high BC. Out of this number for low, normal and high BC, no dog representing 0.0% for low BC, normal BC and high BC were infected (Table 4.15).

4.7 Correlation Analysis of Blood Parameters

Analysis of the blood parameters showed significant difference between the Pack Cell Volume and Haemoglobin (0.897), Pack Cell Volume and Red Blood Cell (0.963), Haemoglobin and Red Blood Cell (0.924), Neutrophil and Lymphocytes (-0.779) with the first three (PCV and HC, PCV and RBC, HC and RBC) showing positive correlation (Table 4.16).

Table 4.15: *Dirofilaria immitis* in Relation to Basophils Count (BC) of the Dogs

Basophils	Number Examined	Number Positive (%)	Chi-square	df	p value
Low	00	0 (0.00)			
Normal	00	0 (0.00)			
High	00	0 (0.00)			
Total	**317**	**(48)**			

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Table 4.16: Correlation Analysis of Blood Parameters

Blood Parameters	PCV (%)	HB (g/dl)	TPP (g/dl)	W.B.C (109/l)	RBC (10/l)	N	L	M	E	B
PCV (%)	1									
HB (g/dl)	0.897**	1								
TPC (g/dl)	0.039	0.106	1							
W.B.C (109/l)	-0.328	-0.318	0.086	1						
RBC (10/l)	0.963**	0.924**	0.058	-0.318	1					
Neutrophil	0.287	0.298	0.236	0.032	0.310	1				
Lymphocyte	-0.271	-0.304	-0.114	0.009	-0.277	-0.779**	1			
Monocyte	0.170	0.143	-0.185	0.068	0.185	-0.062	-0.127	1		
Eosinophil	-0.030	-0.062	-0.333	0.150	-0.033	-0.225	0.014	0.397	1	
Basophil	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion

The prevalence of 15.1 percent of confirmed cases of canine *Dirofilaria Immitis* infection in the population of dogs in Zaria indicates that the disease is not only a common problem in the Zaria areas but likely a threat to public health since dog is a domestic animal living with man at home as the mosquito (vector) have no preference in taking a blood meal. There is a possibility of transmitting the parasite among humans (Anyanwu, 2000).

From the age-specific prevalence of *Dirofilaria immitis* in dog population in Zaria, it can be deduced that younger dogs are more susceptible to the *Dirofilaria immitis* worms than the older dogs. This is probably due to the fact that the puppies lack enough immunological resistance to the filarial worm (Ben-Mahdi, 2009).

The demonstration of a higher *Dirofilaria immitis* infection in the male dogs is probably due to the fact that the male dogs could be more exposed at nights to blood-sucking vectors than the females. This might be possible due to the wandering habits of the male dogs in search of food and mates.

Nevertheless, the detection of microfilaria as a proof of *Dirofilaria immitis* infection is known to go with some drawbacks as about 5 – 20% of dogs infected, microfilaria are not detected in their blood. Although, no microfilaria was detected in the blood when examined, such dogs may be infected. This condition has been explained as an immunologically mediated response in the host for which the microfilaria are

concentrated in the lung tissues and not allowed to circulate in the blood stream of the dog (Sidney, 2004).

Furthermore, the infection rates among the breeds show that, the local breeds are more infected than the mixed breed. Infections were detected in the foreign/Alsatian breed. This may also be attributed to the fact that most of these breeds are kept in cages, under special care in houses or under chains, thus not exposing them to frequent mosquito bites.

The detection of *Dirofilaria immitis* infection among the dogs living in all the 7 location of Zaria likely indicated that the ecological conditions in these areas are similar and favour's the vast breeding of the filarial vectors responsible for the disease transmission. Anyanwu *et al***.** (2000) have implicated *Aedea aegypti, Culex papains* spp, and *Aedes vitattus* mosquitoes as potential vectors of *D. immitis* in Nigeria. This species of mosquitoes are commonly present in our community. This is not also unconnected with the observations of Carsini *et al.* (2007) that dirofilarial infection is determined by the frequency of contact between the dog and the transmitting mosquitoes and of course, the prevalence of disease in the dog population.

In addition, the result also revealed that, stray dogs are more predisposed to the *Dirofilaria immitis* worms than the caged dogs. This is probably due to the wandering habits of the dogs.

The PCV result indicates that at certain stages of infection, the PCV of infected dogs is affected resulting in fewer red blood cells in the body than is expected. These causes weakness and tiredness to the dog since its body would be getting less oxygen than needed (Chaovalit, 2008).

The total protein count was not affected by the presence of the parasite because low total protein count indicate chronic disease (especially liver and kidney disorders), parasitism, long term stress, and starvation or malnutrition. Increases in prevalence may indicate dehydration and chronic infection (Knight,, 2000).

A higher percentage of the positive cases showed higher haemoglobin level, the reason could be that the infection result in the cell containing less than the amount of the haemoglobin and the normal haemoglobin level for a dog is 14 – 20 grams/deciliter.

The WBC count increased in positive cases which is indicative of the dirofilarial infection.

The presence of the infection led to the release of a greater number of white blood cells such as Eosinophils, Lymphocytes, Monocytes and Neutrophils. This corroborates the report of Bionote (2010) which states that an increased WBC means the dog has an infection.

Analysis of the blood parameters showed significant difference between the Pack Cell Volume and Haemoglobin, Pack Cell Volume and Red Blood Cell, Haemoglobin and Red Blood Cell, Neutrophil and Lymphocytes with the first three (PCV and H, PCV and RBC, H and RBC) showing positive correlation while the N and L showed negative correlation (Sidney, 2004).

It is therefore, expected that the filarial parasites can be transmitted to man since dog is a domestic animal living with man at home. As the mosquitoes (vectors) have no preference in taking a blood meal, there is a possibility of transmitting the parasite among the human population.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

A total of 317 dogs were examined, the prevalence of 15.1% were confirmed cases of Dirofilaria immitis in Zaria. 1 – 2 years old dogs has the highest infection rate. The male dogs shows high rate of infection based on sex. Dogs uncaged are highly infected. Infection was recorded in all site/location in Zaria.

The age, sex, breed, home, location, packed cell volume, Neutrophil, Monocyte and Eosinophil showed no significant differences ($P>0.05$).

Furthermore, when white blood cell, lymphocytes, total protein count and haemoglobin concentration in the dogs are low it shows high rate of infection and shows highly significant difference ($P<0.01$) while the red blood cell shows a significant difference ($P<0.05$).

The packed cell volume and haemoglobin, packed cell volume and red blood cell shows positive correlation while Neutrophil and lymphocytes showed negative correlation.

The presence of potential vectors (mosquitoes) *Culex papiens, Aedes aegypti* and *Aedes vitatus* of *Dirofilaria immitis* in Zaria, and a favorable climates that aids its vast breeding coupled with the wandering habit of the dogs (male), exposing it to a mosquito bite which aids the fast spread of *Dirofilaria immitis* in Zaria.

The presence of the vector (mosquito) in Zaria aid the spread of *Dirofilaria immitis* in dogs in Zaria.

6.2 Conclusion

- . The prevalence rate of 15.1% was found in Zaria from a population of 317 dogs.
- The prevalence of *Dirofilaria immitis* was higher in male than in female, higher in 1-2 years age group than the other age group and higher in local breeds than exotic and mixed breeds.

Uncaged dogs showed more positive cases of the Dirofilaria infection than caged/restricted dogs

6.3 Recommendations

It is therefore recommended that preventive measurs have to be taken in order to reduce the high infection rate of the disease in our local pets – the dogs. Such measures include;

1. Control through screening of dogs and therapeutic intervention.
2. Proper enlightenment and education about the disease through health education.
3. People should be encouraged to take their dogs to veterinary clinics for routine examination and possible treatment.
4. Control of known vectors is necessary
5. Early treatment of human cases if detected.

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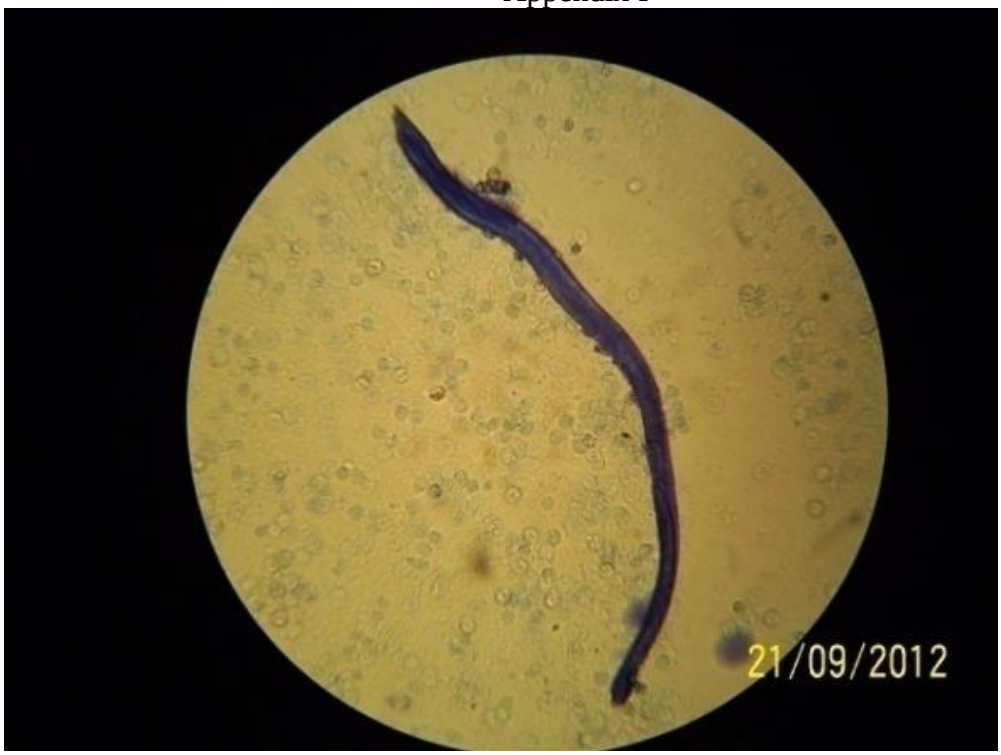
APPENDICES

Appendix I



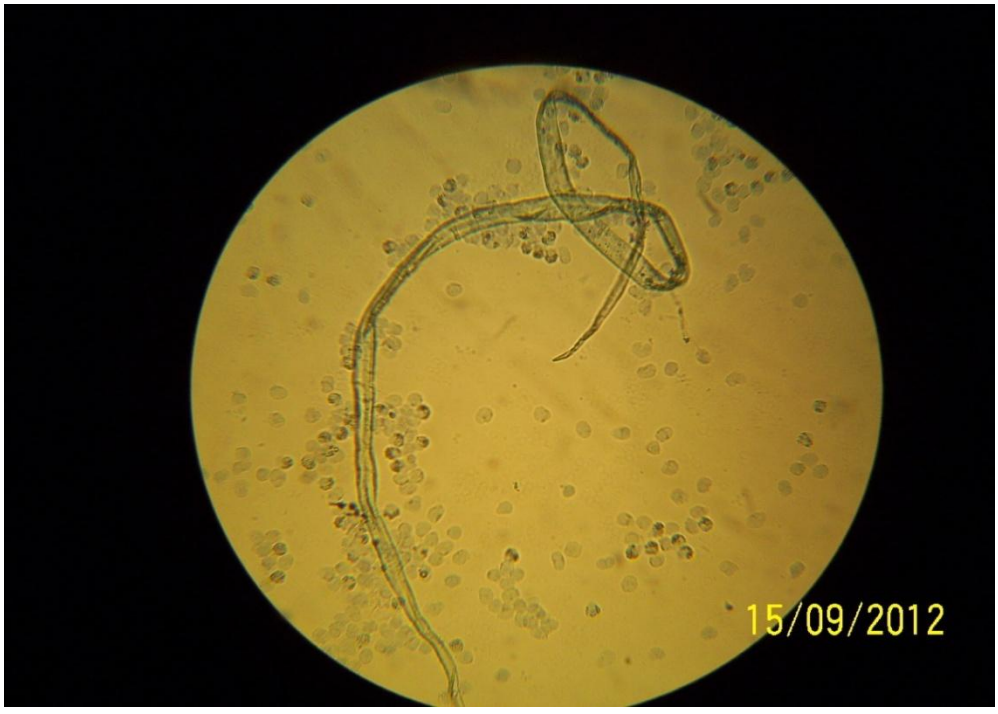


Plate 1: Adult *Dirofilaria immitis* in Dogs

Appendix II

Questionnaire

I am a post graduate student of A.B.U Zaria. I am conducting a research on the prevalence of *Dirofilaria immitis* in dogs in Zaria. The study seeks to enhance prevalence and control of the disease in dogs in Zaria as a case study. I will therefore appreciate your kind assistant in carefully completing this questionaire personally. Every information provided will be handled with at most confidentiality.

- Name of dogs owner (surname first)……
- Residential address.....
- Do you keep dogs at home? Yes ☐ No ☐
- Where is the dog kept? Stray ☐ Home ☐ cage ☐
- Age of the dog? 6 month ☐ 1-2years ☐ 2-4years ☐ 4-7years ☐ 8years&above ☐
- Sex of the dog? Male ☐ Female ☐
- Breed of the dog? Alsatian ☐ Local ☐ Cross ☐ Mixed ☐ Mongrel ☐
- Health habit of the dog? **.....**
- How often do you treat the dogs? Regularly ☐ Sometimes ☐ Never ☐
- Date of last treatment? **.....**
- Type of drugs used for treatment **.....**

Dogs below one year old or receiving any microfilaricide or adulticide were excluded from the study.