PREVALENCE AND CO-INFECTION OF DIROFILARIA AND WEST NILE VIRUS IN DOGS IN KADUNA AND ZARIA METROPOLIS, NIGERIA

BY

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AUGUST, 2018

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BY

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DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,

AUGUST, 2018

DECLARATION

I, hereby declare that the work in this Dissertation titled "**PREVALENCE AND CO-INFECTION OF DIROFILARIA AND WEST NILE VIRUS IN DOGS IN KADUNA AND ZARIA METROPOLISES, NIGERIA**" was carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, under the supervision of Prof. J. kabir and Prof. A. J. Natala. The information derived from this literature has been duly acknowledged in the text and a list of references provided. No part of the Dissertation has been previously presented for another Degree or Diploma in this or any other Institution.

Oluyinka Omolabake Ayoola FASANYA   _____

                                            _____
                                        Signature   Date

CERTIFICATION

This Dissertation titled "PREVALENCE AND CO-INFECTION OF DIROFILARIA AND WEST NILE VIRUS IN DOGS IN KADUNA AND ZARIA METROPOLISES, NIGERIA" by Oluyinka Omolabake Ayoola FASANYA meets the regulations governing the award of the degree of Masters of Science in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. J. Kabir   _____   _____

Chairman, Supervisory Committee   Signature   Date

Prof. A. J. Natala   _____   _____

Member Supervisory Committee   Signature   Date

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Dean, School of Post Graduate Studies   Signature   Date
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DEDICATION

This work is dedicated to God almighty for His grace and mercy upon my life.

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ABSTRACT

Mosquito-borne diseases include diseases caused by viruses (also called arboviruses) and parasites which are transmitted through the bite of an infected mosquito examples of which are West Nile virus, and filariasis. The study was aimed at determining the prevalence of dirofilaria and its co-infection with West Nile virus in dogs attending government based Veterinary clinics and at slaughters in Kaduna metropolis and Zaria. The diagnosis was based on the use of the knotts techniques and Enzyme Linked Immunosorbent Assay. Blood was collected from 30 animals each from 6 government based veterinary clinics and 45 each from 2 slaughter slabs in Kaduna metropolis and 90 from 1 slaughter slab in Zaria giving a total of 360 animals sampled. At each collection unit, the animals were selected using systemic random sampling. Biodata regarding each animal was collected and recorded. Blood collected from each animal was shared into two containers for parasitic and serological analyses. Blood positive for filarial parasites using the knotts technique were analyzed for West Nile virus antibodies using competitive enzyme linked immunosorbent assay (ID-VET). The knowledge of dog owners about mosquito borne infections in animals and humans apart from malaria was studied via the use of questionnaire survey. The results revealed a prevalence of 1.1% (4/360) dogs positive for blood dirofilaria parasite, 30.4% (28/92) of combined positive filarial samples and randomly selected negative samples were seropositive with West Nile virus antibodies and 25% (1) out of the 4 positive filarial samples show co-infection. It was established that a vast majority of dog owners do not know that mosquitoes transmit other diseases apart from malaria and transmission can occur from animals to humans and vice versa.

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CHAPTER ONE

1.0   INTRODUCTION

1.1   Background of the Study

More than 3,400 species of mosquitoes have been recorded worldwide. They include 37 genera, all within a single family, the Culicidae, itself divided into 3 sub-families, Toxorhynchitinae, Anophelinae, and Culicinae (Lane & Crosskey, 1993). They occur in tropical and temperate zones, even above the Arctic Circle but are absent in the Antarctic. They are found as high as 6,000m (above sea level) in mountainous regions and as deep as 1,250 m (below sea level) in caves and mines (Lane & Crosskey, 1993).

Mosquito-borne diseases include diseases caused by viruses (also called arboviruses) and parasites which are transmitted through the bite of an infected mosquito examples of which are blood-borne parasites that cause malaria and filariasis, arboviral infections such as Yellow fever, Zika virus fever, Dengue fever, Chikungunya virus fever, West Nile virus fever and many others. All of these infections are endemic in tropical environments causing recurrent and seasonal epidemics (references). All of them present with mild to severe febrile illness, so are often clinically mistaken. In the recent past surveillance for one of these infections has resulted in the detection of one or more other related infections. Hence syndromic surveillance particularly of fevers of unknown origin is a veritable tool for detection of simultaneous infections or co-circulation of these mosquito-borne pathogens within the same environment and populations (Ioos *et al.,* 2014).

One of such important infections is filariasis, a mosquito-borne, neglected tropical zoonoses with lots of interest locally and internationally and is associated with recurrent fevers.

Filariae are vector-borne parasitic nematodes of which several species are of major public health importance, especially in warm climate countries (Simonsen *et al.*, 2014). Dogs are also commonly infected with filarial parasites. Thus, infections in dogs with the mosquito-transmitted filariae *Dirofilaria immitis* and *Dirofilaria repens* are well known due to severe clinical manifestations elicited in the dogs, but also since some of the vectors are anthropophilic and may cause zoonotic transmission of the infections to humans (Genchi *et al.*, 2007, McCall *et al.*, 2008 and Simon *et al.*, 2009). *Acanthocheilonema reconditum*, *Acanthocheilonema dracunculoides*, *Cercopithifilaria grassi* and *Onchocerca lupi* are other widespread but less known filarial species of dogs (ESCCAP, 2012, Otranto *et al.*, 2013a and Otranto *et al.*, 2013b).

Canine filariasis is becoming increasingly important worldwide not only for its effect on dogs (Adock, 1961; Soulsby, 1978) but more for its zoonotic value (Faust, 1962; Hungerford, 1975).

Uche and Ozunde (1988) in an unpublished observation reported that in most veterinary clinics in Southern Nigeria, usual emphasis on the more common canine diseases like' trypanosomiasis, helminthiasis, babesiosis to mention a few, tends to mask or de-emphasize the importance of some rare but very important diseases like canine filariasis during diagnosis.

It is also not unusual to find veterinarians treating sick dogs, especially in the rural areas, based purely on the clinical signs presented, and their experience of dog diseases in the

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locality without any laboratory examination. This type of practice based on mere historic assumptions and minimal laboratory examination could lead to a wrong diagnosis of rare but very important diseases such as filariasis, which amongst other things could present clinical signs that simulate those of the routine dog diseases in a particular (Uche and Odunze, 1988).

Another disease of resemblance is West Nile fever caused by the West Nile virus (WNV), a mosquito-borne member of the genus *Flavivirus*. The West Nile virus is one of the many members of the genus *Flavivirus* that are known to cause human disease. Birds are the natural reservoir of the virus, which is maintained in nature in a mosquito–bird–mosquito transmission cycle. West Nile virus has been detected in many regions worldwide, including North America, Europe, Africa, the Near East, and Asia (Dauphin *et al.,* 2004). West Nile virus has been shown to cause meningoencephalitis in humans and horses.

West Nile virus is now known to be present globally with the first case in the Western Hemisphere being identified in New York in 1999 (Nash *et al*., 2001). Since then, the disease has occurred with greater frequency in the Southern, Midwestern, and Western states. Symptoms of the infection first appear in the population in early June, with the peak incidence occurring in late August and tapering through early November. The West Nile virus has been reported in Africa, Asia, Europe, the Middle East, and North America. In 1999, the first cases of West Nile virus disease were reported in New York City, and the infection has been spreading throughout the North American continent ever since (Karama and Bernard, 2001). In 2012, a reported 5674 West Nile virus cases

occurred in the United States, the result of a large outbreak of the disease (MMWR, 2013). In 2013, the number of cases was 2469, in 2014, it was 2205, and in 2015, the number was 2060 (Hackethal, 2014; Lindsay *et al.,* 2014).

The life cycle of the West Nile virus involves the microbe's transmission from animals to humans by way of *Aedes, Culex,* or *Anopheles* mosquitoes. The West Nile virus can infect horses, birds, dogs, and other mammals. (Chancey *et al.,* 2015; Peterson and Marfin, 2002; Brinton, 2001). However, wild birds are apparently the optimal hosts for harboring and replicating the virus.

1.2 Statement of Research Problem

The danger posed to the general public by the occurrence of canine dirofilariasis (heartworm infection) in an environment cannot be overemphasized. It is a parasitic nematode that occurs in canine and feline cardiopulmonary system, and it is also the causal agent of human pulmonary dirofilariasis (Vieira *et al.,* 2014). Over 70 species of mosquitoes serve as an intermediate host; *Aedes, Anopheles, Armigeres* and *culex* are the genera acting as vectors (Anderson and Davis, 2014). *Dirofilaria immitis* is a major potentially life-threatening disease of dogs with worldwide distribution and of zoonosis significance in many part of the world (Genchi *et al.*, 2014). *Dirofilaria immitis* is widely distributed in Africa, Asia, Australia, Latin America and Mediterranean countries (Altas *et al.,* 2013). However, *D. immitis* and *D. repens* are also considered agents of parasitic zoonosis in Europe (Johnnes *et al.,* 2013).

A large outbreak of West Nile virus infection in 2012 resulted in 5674 reported cases (51% of which were neuroinvasive), although the number of reported cases for 2014

Comment [WU2]: Begin with the problem statement on dirofilariasis,

dropped to 2205 (61% of which were neuroinvasive). In total, 41,762 cases of West Nile virus were reported to the Centers for Disease Control and Prevention (CDC) between 1999 and 2014, including 18,810 cases of neuroinvasive disease (Hackethal, 2014; Lindsey *et al.*, 2013; CDC, 2015).

The West Nile virus is most commonly identified in Asia, Africa, and the Middle East and is endemic in those parts of the world. In the 1990s, outbreaks of West Nile virus encephalitis were reported in Algeria, the Czech Republic, France, Romania, Russia, and Israel (Petersen and Marfin, 2002). In the Americas, since its introduction into the United States, in 1999, the West Nile virus has spread to Canada and into South America (WHO, 2014).

Reports indicate that less than 1% of persons who are infected with the West Nile virus develop severe illness; of individuals who have severe illness secondary to the infection, 3-15% die.

Severe disease particularly affects the elderly. Advanced age is by far the greatest risk factor for neurologic disease, long-term morbidity, and death, especially in persons older than 75 years. Of the 119 patients who died of West Nile virus in the United States in 2013 (out of 2469 cases), the median age was 78 years (Hackethal, 2014; Lindsey *et al.*, 2013).

The total number of reported deaths from the West Nile virus in the United States between 1999 and 2014 was 1765, including 1641 from neuroinvasive disease (CDC, 2015).

The West Nile virus causes serious manifestations in approximately 1% of persons who are infected, with increased morbidity and mortality in individuals older than 50 years. In hospitalized patients in New York City, neurologic sequelae of the West Nile virus included severe muscle weakness, with approximately 10% of patients developing a complete flaccid paralysis (Asnis *et al*., 2000; Campbell *et al.,* 2002; Nash *et al*., 2001). One in 150 West Nile virus infections results in encephalitis or meningitis, and the mortality rate from severe illness is 3-15%. Individuals older than 75 years are at particular risk (Petersen and Marfin, 2002).

Little information is available concerning the susceptibility of dogs to West Nile Virus infection (Austgen *et al*., 2004). Most dogs spend at least sometime outdoors and thus risk exposure from hematophagus insect vectors (Austgen *et al*., 2004).

1.3   Justification

Mosquitoes are known to transmit malaria and other diseases and are prevalent in all parts of the world. Mosquitoes infected with dirofilaria tend to be better vectors of West Nile Virus. With the presence of a common vector, the chances of transmission of these illnesses are high alongside other mosquito borne diseases.

Knowledge on the distribution, biology and veterinary and medical significance of these filariae is limited, probably due to the less distinct clinical picture seen during infection and to a general lack of diagnostic expertise.

The knowledge of related humans on mosquito-borne diseases, knowledge and expertise of general practitioners, pediatricians and other specialists in Nigeria regarding the nature and management of arbovirus diseases has been shown to be inadequate. Therefore febrile illnesses are generally under or over diagnosed and inappropriate treatment given.

Due to a general non-challant attitude and minimal understanding it is common to find dogs which are kept as pets and security dogs roaming around in bushes and stagnant water bodies exposing them to bites from mosquitoes and all sorts of insects. Much is not known about the West Nile virus in dogs in Nigeria and also the possibility of co-infection with West Nile virus and other arboviruses. This lack of knowledge exposes dog owners and the general populace to the risk of WNV and filarial infections.

Most dogs in Kaduna are used for security and hunting purposes as well as pets and also as a source of food. These dogs spend little time in a mosquito repellant cages and kennels which exposes them to mosquito bites. Hunting dogs come in contact with many mammals which are primary reservoirs of West Nile virus and dirofilariasis. These dogs primarily used as pet animals come in contact and sleep in the houses of owners leading to mosquito bites from animals to humans. There is paucity of information on the presence of West Nile virus and co-infection of West Nile virus and dirofilariasis in Kaduna state Nigeria hence, there is need to investigate the presence of the virus and co-infection with filarial in dogs in Nigeria.

1.4   Aims and Objectives

1.4.1   Aim of the study

To determine the presence and level of Dirofilaria and West Nile virus among dogs attending government based veterinary clinics and at slaughters in Kaduna and Zaria metropolises.

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1.4.2 Objectives

To determine:

1. The prevalence of Dirofilaria infection in dogs attending government-based veterinary clinics and at slaughter in Kaduna and Zaria metropolises.

2. The co-occurrence of Dirofilaria and West Nile virus infections in dogs attending government based veterinary clinics and in those intended for slaughter in Kaduna and Zaria metropolises.

3. The knowledge of dog owners on non-malaria diseases transmitted by mosquitoes.

1.5 Research Questions

(a). How prevalent is filarial and WNV infection in dogs in Kaduna and Zaria metropolises?

(b). How prevalent is co-infection with filarial and WNV infection in dogs in Kaduna and Zaria metropolises?

(c). Are dog owners knowledgeable on non-malarial diseases transmitted by mosquitoes?

<div align="center">**CHAPTER TWO**</div>

2.0   LITERATURE REVIEW

2.1 The Parasite *Dirofilaria*

Dirofilariasis is a disease caused by filarial worms of the genus *Dirofilaria* (Venazzin *et al.*, 2006). Filarial nematodes are common parasites of many vertebrates (Anderson, 2000, Dărăbus *et al.,* 2006). The nematodes of genus *Dirofilaria* belong to family *Onchocercidae* and subfamily *Dirofilariinae* of the order *Spirurida* (Anderson, 2000, Manfredi *et al.,* 2007). This genus consists of 27 valid species, and 15 species of questionable validity (Canestri *et al.,* 1997, Venazzin *et al.*, 2006). The genus *Dirofilaria* is divided in two subgenera. The subgenus *Dirofilaria* includes *Dirofilaria immitis* and the subgenus *Nochtiella* includes *Dirofilaria repens* (Manfredi *et al.,* 2007).

The danger posed to the general public by the occurrence of Canine dirofilariasis (heartworm infection) in an environment cannot be overemphasized. It is a parasitic nematode that occurs in canine and feline cardiopulmonary system, and it is also the causal agent of human pulmonary dirofilariasis (Vieira *et al.*, 2014). Over 70 species of mosquitoes serve as an intermediate host; *Aedes, Anopheles, Armigeres* and *Culex*are the genera acting as vectors (Anderson and Davis, 2014). *Dirofilaria immitis* is a major potentially life threatening disease of dogs with worldwide distribution and of zoonosis significance in many part of the world (Genchi *et al.,* 2014). *D. immitis* is widely distributed in Africa, Asia, Australia, Latin America and Mediterranean countries (Altas *et al.,* 2013). However, *D.immitis* and *D. repens* are also considered agents of parasitic zoonosis in Europe (Johnnes *et al.*, 2013).

2.1.1 Taxonomy

Dirofilariaimmitis belongs to the class Secernnentea, subclass Spiruria, order Spirurida, superfamily Filarioidea, family Onchocercidae. Its scientifically called *Dirofilaria immitis* and commonly called Dog Heartworm (www.smartsite.ucdavis.edu as at 3:26am on the 17/7/2017).

2.1.2 Morphology

Adults are long, white, thread-like worms. Males measure 12-16cm long with the tail spirally coiled. It bears narrow alae and three pairs of large caudal papillae, one of which is postnatal and three pairs of small ones near the tip of the tail. The left pickle is 324-375um long and the right is 90-229um. Females are 25-30cm long with the vulva opening just behind the posterior end of the esophagus. Microfilariae are sheath less, 218-329um long, and have a long-pointed tail (www.smartsite.ucdavis.edu as at 3:26am on the 17/7/2017).

2.1.3   Life cycle of *Dirofilariaimimitis*
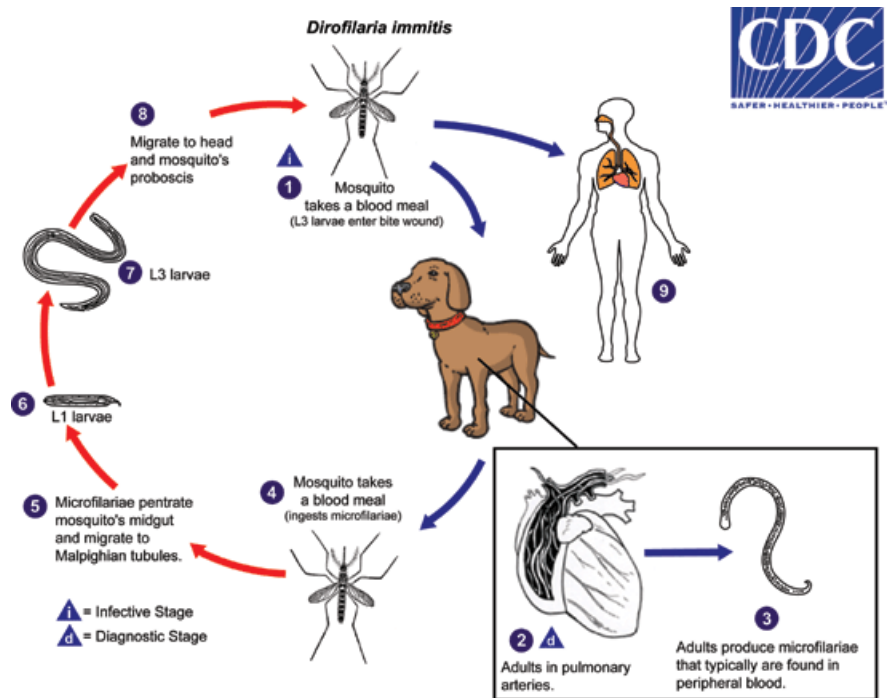
Figure 1: Life cycle image of *Dirofilaria immitis*and information courtesy of *DPDx*.

Adults are parasites mainly of the chambers of the right side of the heart and pulmonary artery. Individual eggs developing in the uterus are enclosed in a thin vitelline membrane. As the embryo elongates, the surrounding membrane stretches to conform as an enclosing sheath. At birth, the membrane is lost and the embryo appears in the blood as a sheath less microfilaria. Microfilariae are deposited in the blood of the chambers at the right side of the heart and pulmonary artery. They are carried through the lungs, into the left chambers of the heart, and into the systemic circulation. There is marked nocturnal periodicity in the peripheral blood. Mosquitoes serve as vectors. After microfilariae are ingested during the blood feeding process, they migrate from the intestine within 24-36 hours into the Malpighian tubules, where further developments and a molt from the first to the second stage juvenile occurs. After 9 days they enter the abdominal hemocoel, where the second molt occurs. Third stage juveniles escape onto the skin and enter it through the feeding site. For about 80 days, juveniles are in the subcutaneous tissues and muscles where the third molt takes place 9-12 days after entry. In the tissues, the fourth stage juveniles attain lengths up to 25mm. they begin entering the right side of the heart shortly after the fourth molt 60-70 days after entering the dog. Development to maturity with males 14-19cm long and females 23-31cm long takes 174-223 days, at which the microfilariae appear in the blood. The reproductive period exceeds 2 years and may extend to 5 (www.smartsite.ucdavis.edu as at 3:26am on the 17/7/2017).

2.1.4 Epidemiology and transmission

In Europe, the most common canine filarial species producing blood-circulating microfilariae are: *Dirofilaria immitis, D. repens, Acanthocheilonema (syn. dracunculoides* and *A. (syn. Dip.) reconditum* (Magnis *et al.*, 2013). Cardiopulmonary

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dirofilariosis or heartworm disease (HWD) is caused by *D. immitis*, which is the one of the most pathogenic parasites of dogs with an increasing distribution, possibly due to climatic changes as well as animal and human movements (Genchi *et al*., 2011). Currently, the distribution of canine dirofilariasis in Africa is not well known due to paucity of epidemiological information, lack of methodological details regarding the assays employed in the methods and the large variety of filarial species in the continent (Simon *et al*., 2012). The presence of *D. Immitis* in dogs in Morocco, Tunisia, Egypt, Tanzania, Kenya, Mozambique, Malawi, Senegal, Angola, Gabon, and Nigeria has been reported. In sierra-Leone, it has been reported in both dogs and cats (Simon *et al*., 2012). In South Africa, autochthonous *D. repens* infections have been documented, where *D. Immitis* infections seem to have been imported (Simon *et al*., 2012). However, Anyanwu *et al*., (1996) reported prevalence of canine filariasis in Zaria, Nigeria to be 8.9% (*D. repens*), 2.5% (*D. immitis* - like parasite), and 4.4% (unidentified species of filarial). Ajadi *et al*. (2011), reported an incident of *D. immitis* in a 3-year-old Dorbaman in Ibadan, Nigeria.

2.1.5 Distribution and prevalence

It has a worldwide distribution but its most commonly found in the mild and warm climates. In the U.S. they are prevalent along the Atlantic and Gulf Coasts (www.smartsite.ucdavis.edu as at 3:26am on the 17/7/2017).

Dirofilaria immitis affects wild and domestic canines and felines and human populations in tropical and temperate regions throughout the world, whereas *D. repens* is exclusive to the Old World. Most of the available epidemiological information originates from a limited number of countries in which these illnesses have been considered both

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and medical concerns for decades. Nevertheless, with increasing frequency, data are being generated in countries where dirofilariasis was not previously documented or for which information was limited. A comparison of the historical epidemiological data in a 10-year period shows that changes in the distribution and prevalence of dirofilariasis are occurring throughout the world. These changes could be partially attributed to the growing interest of the scientific community in dirofilariasis, especially with respect to human infections, and to climate change, which has increased the range of specific vectors of *Dirofilaria* spp. in some regions (Fernando Simón *et al*., 2012).

2.2 The West Nile Virus

West Nile virus (WNV), a mosquito-borne member of the genus *Flavivirus* is a neurotropic human pathogen that is the causative agent of West Nile fever and encephalitis. It causes a worldwide zoonotic infection and its transmitted by mosquitoes. West Nile Virus is described by Bauman (2014) as an example of a generalist virus, which means it has the ability to infect a wide range of cells in many host. West Nile Virus according to Pierson and Diamond (2013), possess the ability to infect and target cells such as monoctyes, macrophages, dendritic cells and neurons. It belongs to the family flaviviridae (Chevalier *et al.,* 2014).

West Nile virus was first identified in 1937 in Uganda in eastern Africa (Smithburn *et al*., 1940). During the late summer of 1999 the virus was introduced into the Western Hemisphere in New York State, when infected individuals were diagnosed (Lanciotti *et al.,* 1999; Marfin and Gubler, 2001). Expansion of the epizootic to 12 states and the District of Columbia took place in the year 2000 (Marfin and Gubler, 2001), and

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currently can now be isolated from many avian and mosquito species throughout North America (Gould and Fikrig, 2004; Granwehr, 2004). From 1999 to 2010, 2.5 million people were reported infected with the virus, with encephalitis and meningitis taking over 12,000 of the reported cases and over 1,300 deaths (Kilpatrick, 2011). It has a broad host range infecting mainly birds and mosquitoes, but also mammals (including humans), reptiles, amphibians and ticks. According to CDC (2016) West Nile virus (WNV) is most commonly transmitted to humans by mosquitoes. It is found in both tropical and temperate regions. It mainly infects birds, but is known to infect humans, horses, dogs, cats, bats, chipmunks, skunks, squirrels and domestic rabbits.

The main route of human infection is through the bite of an infected mosquito. Image reconstructions and cryoelectron microscopy reveal a 45–50 nm virion covered with a relatively smooth protein surface. This structure is similar to the dengue fever virus; both belong to the genus Flavivirus within the family Flaviviridae (Shashikant *et al*., 2009).

2.2.1 Taxonomy
According to CDC, the West Nile virus belongs to the phylum or division Virus, class RNApositive strand virus, family Flaviviridae, genus Flavivirus Japanese Encephalitis Antigenic Complex. Compared to other viruses, members of Flaviviridae West Nile virus tend to be smaller and posses a protein matrix between the virus' capsid and envelope (Bauman 2014).

2.2.2 Morphology

West Nile virus consists of a polyhedral protein coat or capsid contained within a spherical outer envelope, about 50 nanometers in diameter, composed of proteins, lipids and trace metals, and carbohydrates; the capsid is about half the diameter. The capsid surrounds a nucleic acid core of positive sense, single stranded RNA of about 10,000 bases.

In the extra-cellular state, West Nile virions measure around 50 nanometers in diameter and tend to be spherical in shape (Brinton, 2014). More, specifically West Nile is an icosahedron, meaning the virion has a 20 sided protein shell which allow for attachment to hosts' cells and provide protection of the genetic material (Bauman, 2014).

2.2.3 Genetics

The WN virion which is about 45-50 nm in diameter is contained in a host-derived membrane (Schmidt, 2012). The WNV has a positive-sense single strand of RNA as its genetic material which implies that the virus' RNA acts directly on mRNA and instructs ribosomes in the process of protein synthesis, which is between 11,000 and 12,000 nucleotides long; which encode seven non-structural proteins and three structural proteins (Lanciotti, *etal*., 1999). A nucleocapsid is formed from 12 kDa protein blocks and holds the RNA strand; the capsid is contained within a host-derived membrane altered by two viral glycoproteins, the membrane (M) and envelope (E) proteins, embedded in it (Fonseca *et al*., 2009; Schmidt, 2012).

2.2.3.1 RNA and proteins

The West Nile Virus which is an enveloped virion contains a positive single stranded genome. It is in Group IV ((+) ssRNA). The genome is about 11 kb of a single open reading frame (Khromykh *et al.*, 2001). It has been discovered via the use of cryoelectron microscopy that the virus has an icosahedral symmetry and is ~500 Å in diameter. The virus lacks surface projections or spikes (Mukhopadhyay *et al.,* 2003). The virus has a multi-layer organization with the outermost layer corresponding to the E and M transmembrane proteins with a very high density. The core nucleocapsid is made up of copies of the genome RNA and the capsid proteins (Mukhopadhyay *et al.,* 2003). The viral RNA is translated to produce 3 structural and 7 nonstructural proteins. The structural proteins are capsid, envelope, and premembrane proteins and the nonstructural proteins include NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The structural proteins are encoded at the 5' end of the RNA strand and serve the function of virus entry and encapsulation of the genome (Khromykh *et al.*, 2001). The nonstructural proteins serve multiple functions due to the limited number of proteins present in the genome. NS1 does not have an exact role in virion assembly however it has been speculated to help in the replication process (Westway *et al.*, 2002). NS2A, NS2B, NS4A, and NS4B inhibit the innate immune response against viral infection in the body (Liu *et al.*, 2004). NS3 is the protease that cleaves other nonstructural proteins from the polyprotein and encodes enzyme activities (Bazan and Fletterick, 1989). The NS5 protein is essential for viral replication due to the fact that it is the polymerase that encodes a methyltransferase (Koonin, 1993).

Figure 2: An electron micrograph of the West Nile Virus. This image was provided by the Centers for Disease Control and Prevention, part of the United States Department of Health and Human Services. It is a part of the public domain.

2.2.4 Life cycle

The West Nile Virus enters the cell through receptor-mediated endocytosis. DC-SIGNR is a major receptor for this form of endocytosis (Davis *et al*., 2006). Once the virus is internalized in the cell, the virus matures as the pH drops to become slightly acidic within the envelope. Once the endosome has matured the envelope protein undergoes conformational changes where the endocytic membrane fuses with the viral lipid membrane. This causes the release of the viral RNA genome into the cytoplasm of the cell (Modis *et al*., 2004). Once released into the cell the single RNA is replicated and translated into proteins. The viral RNA is replicated by both cellular and viral proteins. The 3 structural proteins expressed from the viral RNA assemble onto the membranes in the endoplasmic reticulum and bud into the cytoplasm by the Golgi network. The virus exits the cell in a lipid envelope through exocytosis when cellular enzymes cleave the prM (Lindenbach, 2007).

2.2.5 Epidemiology and transmission

West Nile virus is one of the most widely distributed of all arboviruses with an extensive distribution in the Old World, throughout Africa, the Middle East, parts of Europe and the former Soviet Union, South Asia, and Australia (Gubler, 2007). The virus had not been detected in North America before the 1999 New York City outbreak. It is unknown how West Nile virus got to the United States; however, the circulating strain is genetically identical to a virus identified in Israel, suggesting importation from the Middle East (Lanciotti *et al*., 1999).

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West Nile Virus is maintained in an enzootic cycle between mosquitoes and birds, but can also infect and cause disease in horses and humans, which serve as incidental dead-end hosts. WNV is endemic in parts of Africa, Europe, the Middle East, and Asia (Dauphin *et al.,* 2004), and following its emergence in the United States in 1999 it has rapidly spread across North America, and has recently been reported in Mexico, South America, and the Caribbean (Deardroff *et al.,* 2006; Komar and Clark, 2006) . Currently, no specific therapy or vaccine has been approved for use against WNV infection in humans.

Donaldieu *et al.* (2013) reported that multiple lineages of WNV have been identified based on the variation of their genomic sequence. Based on this discovery, the quest for more knowledge led to the discovery that two major lineages, lineages 1 and 2, were identified in Africa after the first isolation in Uganda. Lineage 1 is found mainly in central and northern Africa, Europe, Australia and in 1999 emerged in the America. In South Africa and Madagascar Lineage 2 has been found to be and its emergence reported in 2005 in central Europe. Lineages 3 and 4 obtained in Russia, 5 in India and 6 in Spain) are less common lineages and are believed to have likely evolved from separate introductions into the Northern Hemisphere (Rizzoli *et al.*, 2015) as at now discoveries of further lineages are evolving in Africa (Fall *et al.,* 2015). Lineages 1 and 2 are most important from a public health standpoint due to their role in causing epidemics in North America and Europe (Zeller and Schuffenecker, 2004; Hernández-Triana *et al.,* 2014) and are likely under-reported in Africa.

Since the West Nile virus strain (B956) was first isolated from a clinical case of fever in Uganda in 1937 and its introduction in Egypt in 1950 through the present, West Nile

virus has had a significant global public health impact during the last decades due to their resurgence and dynamic epidemiologic features in human and animals, and its considered one of the largest arboviral neuroinvasive disease recorded in the world (Sayed-Ahmed, 2016).

Saka *et* al., (2014) reported that outbreaks of WN virus infections in humans were documented in Algeria, Romania, the Czech Republic, the Democratic Republic of the Congo and Russia with epizootics involving horses occurring in Morocco and Italy.

Documentation of WNV activity in horses in sub-Saharan Africa in Senegal, Côte d'Ivoire, Chad, Democratic Republic of the Congo, Gabon, and Djibouti have been presented (Cabre *etal*., 2006), whereas in South Africa, reports on diagnostic and seroprevalence studies of WNV in horses have been given (Venter *et al*., 2011).

In Nigeria, Olaleye *et al*. (1989) reported that 71% of 62 adult male horses from two stables in Lagos (southwestern Nigeria) were detected with anti-WNV complement-fixing antibodies. Olaleye *et al*. (1990) and Omilabu *et al*. (1989) also indicated WNV to be prevalent among some domestic animals and Baba *et al*. (2006) also indicated the presence of WNV antibodiesas well in humans and mosquitoes in Nigeria. A preliminary study carried out by Saka *et al*. (2014) in Borno state in northern Nigeria revealed that 8.6% of donkeys, 11.5% of horses and 17.7% camels were found to have WNV antibodies.

Following reports of WNV infection among humans in semiarid northeastern Nigeria, Baba *etal*. (2013) carried out a survey on humans in Maiduguri, Borno State, during the

rainy (July to September) and dry harmattan (October to March) seasons from which he obtained a WNV seroprevalence level of 25%.

Epidemiological studies indicated that the frequency and severity of clinical illness increases with age (Nash *et al.,* 2001; Tsai *et al.,* 1998). Infection with WNV remains asymptomatic in the majority of cases or results in West Nile fever (a mild flu-like illness) in approximately 20 to 30% of infected cases (Petersen and Marfin, 2002; Watson *et al.,* 2004). Symptoms are of sudden onset and may include malaise, eye pain, headache, myalgia, gastro-intestinal discomfort and rash (Petersen and Marfin, 2002; Campbell *et al.,* 2002). A small percentage of cases may develop encephalitis, meningitis, or acute flaccid paralysis (AFP) (Tsai *et al.,* 1998; Mostashari *et al.,* 2001; CDC, 2002), and long-term neurological sequelae are common in more than 50% of these cases (Southam and Moore, 1958; Ceausu *et al.,* 1997; Pepperell *et al.,* 2003). Disease manifestation is explained by neuronal damage in several regions of the brain. The fatality rate for hospitalized encephalitic cases is approximately 10%, with increased risk for patients with compromised immune systems, advanced age and with underlying conditions such as diabetes mellitus (Murray *et al.,* 2006).

The proboscis of an *Aedes albopictus* mosquito feeding on human blood. Under experimental conditions, the *Aidesalbolite's* mosquito (also known as the Asian Tiger Mosquito) has been found to be a vector of West Nile virus. The virus is transmitted through mosquito vectors, which bite and infect birds (Hayes *et al.,* 2005). The birds are amplifying hosts, developing sufficient viral levels to transmit the infection to other biting mosquitoes which go on to infect other birds (in the Western hemisphere the

American robin and the American crow are the most common carriers) and also humans. The infected mosquito species vary according to geographical area; in the US *Culex pipiens* (Eastern US), *Culex tarsalis* (Midwest and West) and *Culex quinquefasciatus* (Southeast) are the main sources. In mammals the virus does not multiply as readily (i.e. does not develop high viremia during infection) and it is believed that mosquitoes biting infected mammals do not ingest sufficient virus to become infected, making mammals so-called dead-end infections (Fonseca *et al.,* 2004). *Culex Pipiens* mosquitoes existed in two populations in Europe, one which bites birds and one which bites humans. In North America 40% of *Culex pipiens* were found to be hybrids of the two types which bite both birds and humans, providing a vector for West Nile virus. This is argued to provide an explanation of why the West Nile disease has spread more quickly in North America than Europe. However, these conclusions have been disputed (spielman *et al.,* 2004).

Mosquito bites account for nearly all human infections. West Nile Virus can also be transmitted via transfused platelets, red blood cells, and fresh frozen plasma (Pealer *et al.*, 2003) as well as through heart, liver, lung, and kidney transplants (Nett *et al.*, 2012). Transmission via organ transplant has occurred from donors without detectable viremia, suggesting viral sequestration in organs shortly after viremia has cleared. One possible transplacental transmission following a second trimester infection resulted in an infant with chorioretinitis, lissencephaly, and cerebral white matter loss. Fortunately, fetal abnormalities due to intrauterine infection are uncommon: none of 72 live infants born to 71 women infected during pregnancy had malformations linked to West Nile viral infection or had conclusive laboratory evidence of congenital infection (O' Leary *et al.*, *2006).* Nevertheless, 3 neonates born to women infected within 3 weeks' prepartum

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developed symptomatic West Nile virus disease at or shortly after birth, indicating the possibility of intrauterine infection or infection at the time of delivery. Other rare or suspected modes of transmission include breast milk transmission, percutaneous or conjunctival exposure to laboratory workers, and by unknown means in patients undergoing dialysis and workers at a turkey breeder farm (Petersen and Hayes, 2008).

2.2.6 Distribution and prevalence

West Nile Virus is commonly found in North America, Europe, Africa, the Middle East, and West Asia (IAMAT, 2018).

2.3 Dirofilariasis in Animals

Dirofilaria immitis can cause a severe cardio-pulmonary disease in dogs and cats and pulmonary dirofilariosis is also a rare zoonosis (McCall *et al.,* 2008).Symptoms in dogs appear when more than 25 worms are present. Up to 60 worms cause circulatory difficulty. With 100 or more worms, there is blockage of the pulmonary artery and right side of the heart, accompanied by interference with the heart valves. The right side of the heart becomes dilated and enlarged. Blood backs up in the liver and other parts of the body, causing general congestion and degeneration. Dogs fatigue easily, cough, and appear unthrifty (www.smartsite.ucdavis.edu as at 3:26am on the 17/7/2017).

2.3.1 Clinical signs of dirofilariasis in animals

The clinical signs of the disease include coughing, exercise intolerance, unthriftiness, dypsnea, cyanosis, hemoptysis, syncope, epistaxis and ascitis may develop due to right sided chronic heart failure (Camillie-Marie *etal*., 2015). A large number of infected dogs

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had abnormalities in their hematological and biochemical profile. Clinical hematology study showed mild to moderate anemia in microfilaremic dogs (CAPC, 2015).

2.3.2 Pathogenesis of dirofilariasis in animals

The primary damage in heartworm infection occurs in the pulmonary arteries and lungs. The degree of damage depends on the number of worm's present, the duration of infection, and the host's reaction to the parasites' presence. It is believed that the L5 heartworms cause the damage when they reach the pulmonary artery (3 months after infection) (Atkins, 2005). The immature adult worms initiate vascular damage and possibly lung disease by causing eosinophilia with eosinophilic infiltrates and signs of respiratory disease (Atkins, 2005). The adult worms typically live in the caudal pulmonary vascular tree, where they cause further damage through the release of toxic substances, the host's own immunologic reaction to these substances, and physical trauma. The initial vascular changes include endothelial damage and sloughing, villous proliferation, and activation and attraction of leukocytes and platelets. These changes may eventually lead to smooth muscle cell proliferation and collagen accumulation, resulting in fibrosis. Dead or dying worms cause the most severe damage, including thrombosis, granulomatous inflammation, and rugose, villous inflammation. Affected vessels may become thrombosed, thickened, dilated, tortuous, noncompliant, and functionally incompetent.

Heartworms release vasoactive substances that result in vasoconstriction and hypoxia, which lead to pulmonary hypertension and compromised cardiac output (Kith*et al.,* 2001). Pulmonary hypertension causes pressure overload of the right ventricle, resulting

in compensatory, concentric ventricular hypertrophy (thickening of the ventricular walls). In severe cases (high worm burdens or chronic infections), chronic pulmonary hypertension with tricuspid insufficiency results in elevated cardiac filling pressures and congestive heart failure. Thromboembolism may cause acute decompensation by producing or aggravating pulmonary hypertension, right heart failure, or pulmonary infarction. Therefore, dead worms tend to worsen the vascular damage and enhance coagulation.

The pulmonary parenchyma can also be damaged. Eosinophilic pneumonitis is the most commonly reported parenchymal lesion and is caused by immune-mediated destruction of microfilariae within the pulmonary vasculature and the subsequent inflammatory reaction (Calvert and Losonsky, 1985). Much less commonly reported is pulmonary eosinophilic granulomatosis, which develops when microfilariae trapped within the lungs are surrounded by neutrophils and eosinophils, leading to granuloma formation. The most severe manifestation of heartworm disease is caval syndrome, in which a percentage of the worm burden is redistributed to the right ventricular inflow tract, resulting in severe tricuspid regurgitation and decreased forward flow. Hemolytic anemia, secondary to traumatic destruction of the red blood cells as they pass through the worm mass, also occurs. This intravascular hemolysis leads to hemoglobinuria (Strickland, 1998). Some patients with caval syndrome present with clinical signs referable to right-sided congestive heart failure.

Heartworm infection may also lead to glomerulonephritis and proteinuria secondary to antigen-antibody complex formation. However, this does not commonly lead to renal

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failure (Grauer, 2003). Heartworms can also produce disease by means of aberrant migration in tissues such as the brain, spinal cord, eye, liver, or skin. The resulting depend on the path of migration.

Heartworm-infected cats usually have low worm burdens (two to four worms) (Mccall, 1992). As in dogs, immature adults may induce pulmonary vascular disease before maturation. These changes may develop in cats that resist mature infection (Atkins, 2005). The pulmonary arterial response to adult heartworms is more severe than that in dogs. Cats have a smaller pulmonary arterial tree with less collateral circulation, making them more susceptible to worm embolization (Atkins, 2005). The clinical signs associated with the presence and death of heartworms within the pulmonary arteries in cats have become part of a syndrome known as *heartworm-associated respiratory disease*. Rarely, heartworm infection in cats may lead to right heart failure, resulting in pleural (may be chylous) effusion, ascites, or both.

2.4 Dirofilariasis in Humans

Human dirofilariasis is an infection caused by filarial nematodes of the genus *Dirofilaria* usually transmitted by mosquitoes from carnivorous mammals to humans. There are different forms of clinical manifestation. Man is occasionally infected, the disease either manifests itself as subcutaneous nodules or as pulmonary coin lesions (Stemberger, 1986).

2.4.1 Clinical signs of dirofilariasis in humans

Human pulmonary dirofilariasis is characterized by the formation of pulmonary nodules (Fig. 3) around immature adult worms that have recently molted from L4 larvae. When L4 larvae reach a small or medium branch of the pulmonary artery, they block its passage, causing embolism and localized inflammation (Muro and Codero, 2001). The most significant event with far-reaching consequences in human pulmonary dirofilariasis management is that the discovery of such nodules is frequently misdiagnosed as a malignant lesion (Simon *et al.,* 2005). Gross histology reveals a central clot, which often traps a worm, surrounded by a yellowish or whitish fibrous wall 1 to 3 mm thick. In many cases, histopathology exposes worm structures at various stages of decomposition in the arterial lumen, surrounded by copious inflammatory infiltrates. In some cases, only a cellular reaction is observed because worms have already been destroyed by the time the nodule is found (Simon *et al.,* 2005). Histological studies of lung nodules caused by *D. immitis* have shown that such cellular infiltration comprises eosinophils, lymphocytes, and plasma cells, accompanied by a histiocytic reaction and inflammatory changes in the tissues surrounding capillaries. These events are responsible for nodule formation rather than infarction stemming from embolism formation. Necrotic regions with pulmonary artery disruption due to exiting worms are also frequently observed (Araya *et al.,* 2007).

Although single nodules appear most frequently, multiple lesions have also been described, with a maximum of five occurring in the same individual (Kocher, 1985). In general, radiological characteristics—spherical or ovoid nodules with well-defined borders and a homogeneous density—suggest a benign profile (Muro and Codero, 2001). Residual calcified lesions have also been described, which are consistent with the

angiocentric lesions typical of dirofilariasis (Codero *et al.,* 1992). In previous reports, times to nodule formation of 2, 3, and 8 months have been reported (Khan, 1983, Kocher, 1985, Navarrette, 1972). To date, the longest-known residence of a single nodule is 13 years (Beskin *et al.,* 1976), during which the nodule underwent calcification, whereas a 2-year follow-up of a single nodule did not reveal any modifications to its radiological features (Muro and Codero, 2001). These lesions often disappear with time, suggesting that pulmonary dirofilariasis can present with transient lesions (Codero *et al.,* 1992).

There is evidence that nodules tend to be most frequently found in the right lung although with no differences in lobar distribution. Nodules are commonly found in peripheral locations, usually in subpleural regions. Pulmonary dirofilariasis is detected at the highest frequencies in male adults with a mean age of 53 years, although infected patients range in age from 10 to 79 years (Muro and Codero, 2001). Only a small number of patients present with symptoms associated with pulmonary dirofilariasis. When these symptoms do arise, they are nonspecific and include coughing with pleural or nonpleural thoracic pain (at comparable frequencies), purulent or hemoptoic sputum with hemoptysis and dyspnea in the minority of cases, fever, and other nonspecific signs such as malaise and myalgia. Only one case was initially diagnosed as a pulmonary embolism. Nevertheless, this diagnosis is considered a possible cause of the symptoms in most symptomatic cases that show nodules on an X-ray. Auscultation is almost always normal, with crepitation, stertor, and wheezing being the most frequent signs among abnormal profiles. When present, pleural effusion is of a low magnitude (Muro and Codero, 2001).
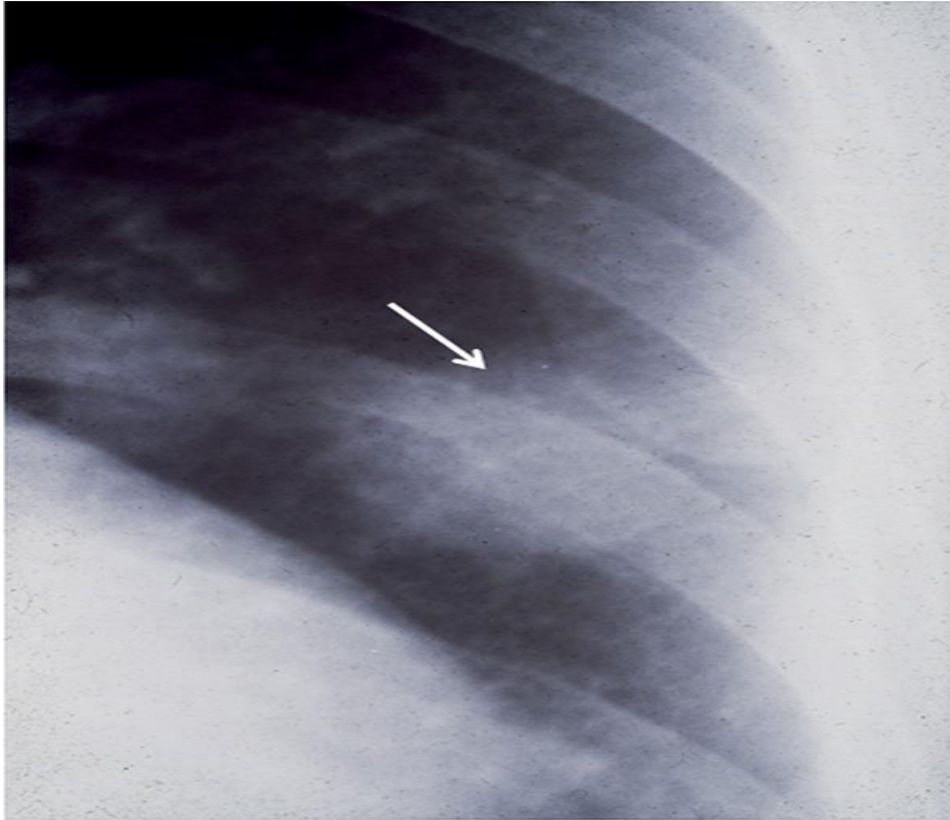
Figure 3: Human pulmonary dirofilariasis. Shown is a thoracic radiograph showing a

pulmonary nodule attributed to *D. immitis* (arrow).

2.4.1.1 Subcutaneous/ocular dirofilariasis.

Subcutaneous dirofilariasis, which is caused by adult and preadult *D. repens* worms in subcutaneous tissues, presents as a subcutaneous nodule (Fig. 4A and B) B that grows gradually over a period of weeks or months. It has a firm, elastic consistency and is associated with erythema. Histology reveals four types of nodules, with diverse contents and characteristics (Pampiglone and Rivasi, 2007). Although the highest incidence of subcutaneous cases occurs in individuals aged 40 to 49 years, infections in patients of all ages have been described, most notably in Sri Lanka, where 33.6% of the reported infections have occurred in children under 10 years of age. In contrast to pulmonary dirofilariasis, women seem to be more susceptible to subcutaneous dirofilariasis than men (55.4% versus 44.6%).

The percentage of reported cases of ocular dirofilariasis has been increasing in recent years. Between 30% and 35% of *D. repens*-related infections occur in ocular regions (orbital zone, eyelids, and subconjunctival and intravitreous tissues) (Fig. 4C) (Genchi *et al.,* 2011, Pampiglone and Rivasi, 2000). Some of these cases have serious consequences, with symptoms including damaged vision, floaters, or loss of sight (Genchi *et al.,* 2011). Permanent complications, such as retinal detachment, glaucoma, opacity of the vitreous humor, crystalline lens, or other losses of visual acuity, will develop in 10% of patients (Avdiukhina, 1996). Additional risks and side effects are associated with the surgical extraction of worms from sensitive areas, such as the optic nerve (Korkhov *et al.,* 2009). In cases with orbital localization, symptoms such as blepharedema, palpebral ptosis, and moderate ocular discomfort occur (Stringfellow *et al.*, 2000). Worms in the ocular conjunctiva can also cause inflammation in addition to hyperemic conjunctival tumefaction (Ruiz-onero *et al.,* 1998).
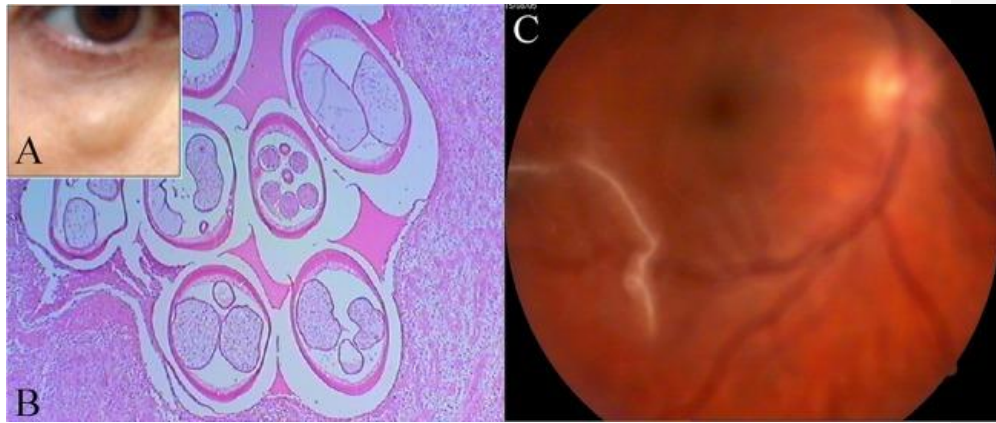
Figure 4: Human subcutaneous and ocular dirofilariasis. (A) External appearance of a subcutaneous nodule in the ocular region. (B) Histological section of a nodule showing sections of adult *D. repens* worms. (C) Intravitreal location of an adult *D. repens* worm in a human patient. (Panels A and B courtesy of Vladimir Kartashev, University of Rostov Na Donu, Rostov, Russia; panel C reprinted from reference (Korkhov *et al.,* 2009)

2.5 West Nile Virus in Animals

West Nile virus (WNV) primarily affects birds, but can also be infect bats, horses, cats, dogs, chipmunks, skunks, squirrels, domestic rabbits, alligators and humans (CFSPH, 2013). WNV infections have been documented in sheep, cattle and pigs in Africa and Eurasia (CFSPH, 2013). Most infections are without clinical signs and animals developed antibodies to the virus. However, in laboratory studies sheep infected with WNV were reported to have exhibited fever, abortion and encephalitis (ct.gov, 2013).

2.5.1 Clinical signs of west nile virus in animals

Most animals infected with WNV show no signs of illness. Infected horses may develop encephalitis and have lethargy, weakness, incoordination, paralysis or even death. Most do not become febrile. Death may occur in birds. A very small number of dogs have been infected with the virus but generally show few signs of illness (cdc.gov, 2017). Birds are usually not known to show any clinical signs when infected with WNV. Chickens upon infection with WNV usually do not become sick. In domestic pets such as dogs and cat's signs of fever, depression, incoordination, muscle weakness or spasms, seizures or paralysis may be an indication of West Nile virus infection (cdc.gov, 2017).

2.5.2 Pathogenesis of west nile virus in animals

Following peripheral inoculation, initial WNV replication is thought to occur in skin Langerhans dendritic cells (Bryne *et al.,* 2001). These cells migrate to and seed draining lymph nodes, resulting in a primary viremia and subsequent infection of peripheral tissues such as the spleen and kidney. By the end of the first week, WNV is largely cleared from the serum and peripheral organs and infection of the CNS is observed in a

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subset of immunocompetent animals. Rodents that succumb to infection develop a CNS pathology similar to that observed in human WNV cases, including infection and injury of brain stem, hippocampal, and spinal cord neurons (Fratkin *et al.,* 2004; Eldadah and Nathanson, 1967). WNV infection is not significantly detected in nonneuronal CNS cell populations in humans or animals. In surviving wild-type mice, WNV is cleared from all tissue compartments within 2 to 3 weeks after infection. However, persistent viral infection in the brains of CD8^{+} T-cell (Shrestha and Diamond, 2004) - or perforin-deficient mice (Shrestha *et al.,* 2006) and in the brains and kidneys of infected hamsters has been reported (Tesh *et al.,* 2005; Xiao *et al.,* 2001). Persistent infection has also been documented in a WNV-infected immunosuppressed patient in whom viremia was detected for more than 60 days (Brenner *et al.,* 2005).

2.6 West Nile Virus in Humans

Most people infected with West Nile virus either don't develop signs or symptoms or have only minor ones, such as fever and mild headache. However, some people develop a life-threatening illness that includes inflammation of the spinal cord or brain.

Mild signs and symptoms of a West Nile virus infection generally go away on their own. But severe signs and symptoms — such as a severe headache, fever, disorientation or sudden weakness — require immediate attention. Most people infected with the West Nile virus have no signs or symptoms.

In less than 1 percent of infected people, the virus causes a serious neurological infection, including inflammation of the brain (encephalitis) and of the membranes surrounding the brain and spinal cord (meningitis). (www.mayoclinic.org as at 4:57am 10/6/18)

If you have West Nile virus, you will typically show the first virus symptoms within to 14 days of being bitten. (www.mayoclinic.org as at 4:57am 10/6/18)

Signs and symptoms of West Nile fever usually last a few days, but signs and symptoms of encephalitis or meningitis can linger for weeks or months. Certain neurological effects, such as muscle weakness, can be permanent.

 (www.mayoclinic.org/diseases/westnilevirus as at 4:57am 10/6/18).

2.6.1 Clinical signs of west nile virus in humans

West Nile Virus (WNV) has three different effects on humans. The first is an asymptomatic infection; the second is a mild febrile syndrome termed West Nile Fever; the third is a neuroinvasive disease termed West Nile meningitis or encephalitis. In many infected individuals the ratio between the three states is roughly 110:30:1 (Olejinik, 1952). The second, febrile stage has an incubation period of 2 to 8 days followed by fever, headache, chills, diaphoresis (excessive sweating), weakness, lymphadenopathy (swollen lymph nodes), drowsiness, pain in the joints and symptoms like those of influenza or the flu. Occasionally there is a short-lived truncal rash and some patients experience gastrointestinal symptoms including nausea, vomiting, loss of appetite, or diarrhea. All symptoms are resolved within 7 to 10 days, although fatigue can last for some weeks and lymphadenopathy can take up to two months to resolve. The first signs are swelling (Smithburn and Jacobs, 1042). The more dangerous encephalitis is characterized by similar early symptoms but also a decreased level of consciousness, sometimes approaching near-coma. Deep tendon reflexes are hyperactive at first, later diminished. There are also extrapyramidal disorders. Recovery is marked by a long

convalescence with fatigue. More recent outbreaks have resulted in a deeper study of the disease and other, rarer, outcomes have been identified. The spinal cord may be infected, marked by anterior myelitis with or without encephalitis. WNV-associated Guillain-Barre syndrome has been identified and other rare effects include multifocal chorioretinitis (which has 100% specificity for identifying WNV infection in patients with possible WNV encephalitis), hepatitis, myocarditis, nephritis, pancreatitis and splenomegaly (Ahmed *et al.,* 2000).

2.6.2 Pathogenesis of west nile virus in humans

WNV is thought to replicate at the site of inoculation and then spread to lymph nodes and the bloodstream (Diamond *et al*., 2003). Viral penetration of the central nervous system appears to follow stimulation of toll-like receptors and increased levels of tumor necrosis factor-α, which increases permeability of the blood-brain barrier (Wang *et al*., 2004). WNV directly infects neurons, particularly in deep nuclei and gray matter of the brain, brainstem, and spinal cord (Kleinschmidt-DeMaster *et al*., 2004). Collateral destruction of bystander nerve cells may contribute to paralysis (Darman *et al.,* 2004). Immune-mediated tissue damage may also contribute to pathologic changes in some cases (Leis and Stokic, 2005). Genetic susceptibility for severe disease in mice has been postulated to involve a deficiency in production of $2'-5'$oligoadenylate synthetase, but this genetic susceptibility has not been elucidated in humans (Ceccaldi *et al.,* 2004). Although most nonfatal WNV infections appear to be cleared by the host immune response, the virus may persist in some vertebrate hosts (Ceccaldi *et al.,* 2004; Kuno, 2001).

WNV is maintained in an enzootic cycle between mosquitoes and birds (Hayes *et al.,* 2005) but can also infect and cause disease in other vertebrate animals, including horses

and humans. In most humans, WNV infection is subclinical, but approximately 20%−40% of those infected may develop symptoms of WNV disease ranging from West Nile fever (fever, headache, malaise, lymphadenopathy, myalgia, fatigue, skin rash, diarrhoea, and vomiting) to meningoencephalitis (muscle weakness, tremors, paralysis, and cognitive impairment) or flaccid paralysis (a polio-like syndrome),and, less frequently, death (Karmer *et al.,* 2007; Hayes and Guber, 2006; Leis *et al.,* 2003; Petersen and Marfin, 2003). Hepatitis, pancreatitis, and myocarditis have also infrequently been described to occur (Karmer *et al.,* 2007). In addition, long-term sequelae, including weakness, persistent movement disorders, and cognitive deficits, frequently occur in patients that have suffered from West Nile neuroinvasive disease (Cao *et al.,* 2003; Sejvar, 2007). Although inactivated and recombinant vaccines are available for animal use, no vaccines or antiviral therapies are currently approved for humans (De Filite, 2012).

2.7 Diagnosis

2.7.1 Diagnosis of dirofilariasis

Diagnosis of Dirofilariasis in companion animals is mainly performed by; modified Knott's technique, microfilarial density test, x-ray or ultrasound and commercial serological test such as SnapR, Idexx, DiroCHEKR, Agen, WitnessR (Altas *etal*., 2013). The diagnosis of *D. immitis* is based on detection of circulating antigen or microfilariae released by mature adult female worm into the blood circulation, both being detectable as from 5 and 6-month post infection respectively (CAPC, 2015).

2.7.2 Diagnosis of west nile virus

Detection of IgM antibody in serum or cerebrospinal fluid (CSF) using the IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) forms the cornerstone of West Nile virus diagnosis in most clinical settings. Because IgM antibody does not cross the blood-brain barrier, its presence in CSF indicates CNS infection. At least 90% of patients with encephalitis or meningitis have demonstrable IgM antibodies in CSF within 8 days of symptom onset. The West Nile virus–specific IgM antibody may not be detected initially in serum or plasma; 1 study showed that only 58% of patients with West Nile fever had a positive MAC-ELISA result at clinical presentation (Tilley *et al.,* 2006). Nevertheless, MAC-ELISA testing of acute and convalescent-phase sera will provide definitive diagnosis. Testing for IgG antibodies has no utility in the acute clinical diagnostic setting.

2.8 Treatment

2.8.1 Treatment of dirofilariasis

Infection in dogs can be treated by administration of both ivermectin and doxycycline for several months prior to melarsomine dihydrochloride or possibly even without melarsomine, will eliminate adult heartworm with high risk of severe thromboembolism than melarsomine alone and will block transmission of the parasite (Vieira *et al*., 2014).
In humans, Dirofilariasis is treated with surgical removal of lung granulomas and skin nodules (CDC, 2012; Alena, 2015).

2.8.2 Treatment of west nile virus

AMD3100, which had been proposed as an antiretroviral drug for HIV, has shown promise against West Nile encephalitis. AMD standing for AnorMeD (which was originally called JM3100) is a derivative of the original bicyclam, JM1657 (JM standing for Johnson Matthey) (De Clarke, 2015). It inhibits the CXCR4 chemokine receptors on CD34+ cells and reversibly blocks binding of the ligand, stromal cell-derived factor-1-alpha (SDF-1α) (Hatse *et al*., 2002). By blocking the interaction between SDF-1α and CXCR4 with plerixafor, mobilization of progenitor cells is triggered. Filgrastim, a granulocyte-colony stimulating factor, is added to enhance CD34+ cell mobilization, thus increasing the yield of stem cells- an important determinant of graft adequacy (Hatse *et al*., 2002). Morpholino antisense oligos conjugated to cell penetrating peptides have been shown to partially protect mice from WNV disease (Deas *et al*., 2007). There have also been attempts to treat infections using ribavirin, intravenous immunoglobulin, or alpha interferon. GenoMed, a U.S. biotech company, has found that blocking angiotensin II can treat the "cytokine storm" of West Nile virus encephalitis as well as other viruses (Brave *et al*., 2010). In 2007 the World Community Grid launched the Discovering Dengue Drugs – Together project. This uses a distributed network of volunteers' computers via the Berkeley Open Infrastructure for Network Computing (BOINC) to perform computer simulations of interacting molecules. Thousands of small molecules are screened for potential antiviral properties with respect to West Nile and related viruses (Moskowitz and Johnson, 2004).

2.9 Prevention and Control

Prevention and control is achieved through mosquito control via physical, biological and chemical control methods. Physical control methods are the most ancient methods which have stood the test of time and still remain the best and safest methods of mosquito control. They include use of mosquito nets screens at doors and windows of buildings to prevent mosquitoes from entering buildings, elimination of mosquito breeding sites through activities such as clearing refuse dump sites, eliminating dirty stagnant water bodies, clearing and burning bushes, clearing of gutters and drainage systems (Shashikant *et al.,* 2009).

Chemical control methods include the use of pesticides. This method provides immediate short term relief. The use of pesticides and insecticides is a more practical approach to handle massive mosquito outbreaks thereby reducing the threat of new disease outbreaks but with a massive disadvantage of chemicals which can be harmful to the human health. Another disadvantage of the use of insecticides is resistance to chemicals after a period of time (Olson, 1979).

The use of mosquito repellents containing DEET is another form of chemical control which should be encouraged due to the fact that a lot of time is spent in the outdoors.

In addition to the time spent outdoors and the use of mosquito repellants, long covering clothing should be worn.

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CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1   Study Area

This study was carried out in locations in Kaduna and Zaria metropolises. Kaduna state which is located in the North-West Zone of Nigeria, it is located between Longitude E006.5^{o} – E008.6^{o} and Latitude N09.2^{o} – N11.3^{o}. The state shares boundaries with Niger State to the West, Zamfara, Katsina and Kano States to the North, Bauchi and Plateau to the East, FCT and Nasarawa State to the South. The state occupies an area of about 46,063 square kilometers, (Jallo, 2000). Kaduna State has a projected human population of 6,066,562 (National population census, 2006).

Kaduna State has a total annual rainfall varying between 1,000mm and 1,500mm and a rainy season, which is between 120-150 days long. The Northern part of the State is semi-arid, further South as the rain level increase, the climate becomes sub-humid. The extreme South of the state is marked by a series of rocky hills, which are responsible for the island of rainfall, (Northern Livestock Report, 1992). The state is essentially an agrarian society with about 75% of the populace engaging in farming and it also has potentials for livestock production.

Zaria is a major city in Kaduna State in northern Nigeria, as well as being a Local Government Area. Formerly known as Zazzau. Its located between coordinates 11°04N and 07°42E with an population estimated of 408,198 as at 2006 population census.

It lies within the tropical wet/dry climatic zone and is characterized by a strong seasonality in rainfall lasting from April to September and a drier season from October to March and temperature distribution with warm weather year-round, a wet season, (Umar, 2012).

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Figure 5: Map of Nigeria showing Kaduna State. This image was provided b

African Prime News

Figure 6: Map of Kaduna State showing Zaria. This image was provided by

Research-gate
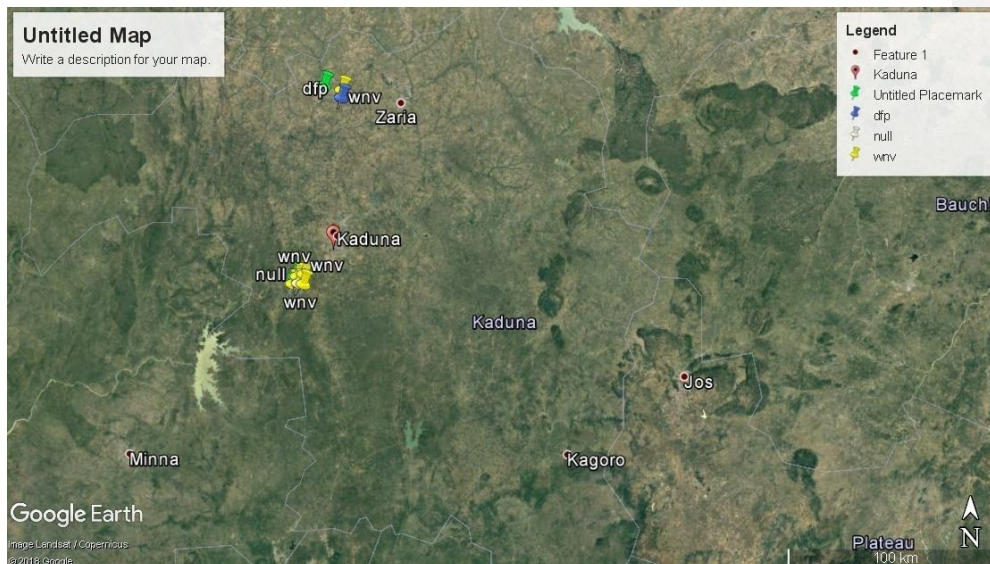
Figure 7: Map of Nigeria showing Sample locations and results obtained from this study.

3.2 Study Design

The study was a cross-sectional descriptive study that involves survey for Dirofilaria and WNV infection in dog population and a questionnaire based survey of dog owners.

3.3 Determination of Sample Size

Sample size was determined using the method described by Thrushfield (2012) using the formula for calculating sample size for cross-sectional studies. For the two infections, a prevalence of 12.7% (Anyawu *et al*., 1996) for canine dirofilariasis was used. Questionnaires were administered to owners of dogs sampled.

3.4 Sample Collection and Processing

The dog population sampled were all dogs from 7 months and above presented to the clinics and for slaughter based on owner's consent. The sampling frame was a list of all government based veterinary clinics and slaughter slabs in Kaduna and Zaria obtained from ministry of Agriculture. Dogs were sampled from all the four government based veterinary clinics in Kaduna and the two clinics in Zaria; as well as the two available slaughter slabs in Kaduna and the one in Zaria. The primary sampling units were the individual dogs for parasitological and serological survey and dog owners for the questionnaire survey. The inclusion-exclusion criteria were dogs from 7 months of age whose owners consented and could be restrained. The clinics visited include:

Magajin gari veterinary clinic Kaduna

Tudun wada veterinary clinic Kaduna

Unguwan rimi veterinary clinic Kaduna

Sabo veterinary clinic Kaduna

ABU veterinary teaching hospital Zaria

Kofar doka veterinary clinic Zaria

The slaughters visited include:

Television garage slaughter Kaduna

Trikania slaughter Kaduna

Zaria slaughter slab.

3.4.1 Sampling procedure

All government veterinary clinics consisting of 4 clinics in Kaduna and 2 clinics in Zaria metropolis, two slaughter slabs randomly selected in Kaduna metropolis and the only slaughter slab in Zaria were used for the study. Dogs whose owners consented were sampled. In cases were by multiple dogs were present 2 of the dogs that cooperated were randomly picked. The sampling was done during the period between (October 2016- April 2017). Samples were collected from dogs from ages 7months upward.

Each clinic was visited and dogs that were brought into the clinic for routine check up, vaccination, treatment was sampled. Dogs registered with each clinic but were not brought in were visited in their homes were also sampled. Each dog was checked for the presence of ectoparasites and the age, sex, breed, purpose for which the dog was kept and body condition score of each dog was recorded using the Nestle Purina score system

(<u>Laflamme, 1997</u>). The selected slaughters were visited at random when dogs were available for slaughter. Each dog was checked for the presence of ectoparasites and the age, sex, breed, purpose for which the dog was kept and body condition score of each dog was recorded.

3.4.2   Sample handling and processing

Five millilitres of blood was collected aseptically from the jugular vein of each dog by a trained veterinarian doctor and aliquoted into EDTA and non EDTA containers. Samples were labeled and transported in a cold box to the viral Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, where blood for serology was centrifuged at 3000rpm for 5mins (Ghazei, 2006). The sera was harvested, labeled and preserved at -20°C until needed for further analysis. Whole blood was processed immediately for haematological and parasitological analysis.

3.4.3   Laboratory analysis

3.4.3.1 Examination of blood for microfilariae

Blood was collected in capillary tubes and spun in a centrifuge and PCV and Hb readings were taken.

Blood was examined for microfilariae using Knott's concentration technique (Knott, <u>1939</u>; Magnis *et al*., <u>2013</u>; Melrose *et al*., 2000 and Genchi *et al*., 2007). In the method 1ml of whole blood was mixed with 9ml of 2% formalin in a conical centrifuge tube and then centrifuged for 5min at $692 \times g$. The supernatant was decanted by inverting the tube.

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The deposit was mixed with one drop of 1% methylene blue stain. 10μl of the sample was transferred to a glass slide and examined under a compound microscope at 100× to identify the microfilariae. Any sample where atleast one microfilaria was seen was considered positive. Circulating microfilariae were identified based on morphology (Magnis *et al*., 2013). Microfilariae were identified morphologically via the use of laboratory charts.

3.4.3.2 Serological analysis of samples for west nile virus infection.

The presence of West Nile Virus antibodies as evidence of WNV infection was determined in samples found to be positive for filarial infection and an equal number of negative random samples from each sampling location using a competitive enzyme linked immunosorbent assay (ELISA) kit (ID Screen WNV Competitive Multi-Species, ID-VET, France) for the testing dogs for evidence of infection with WNV.

The test was carried out using 96 microtitre plates competitive enzyme linked immunosorbent assay (ID-VET). All the reagents were allowed to come to room temperature before use

Fifty microliter (50ul) of dilution Buffer 2 (A complex protein solution) was added to each micro well and 50ul of the positive control, (dilution of a positive horse serum in a stabilizing buffer) was then added to wells A1 and B1 which contains a purified WNV anti-Pr-E antibody (standard).

Fifty microliter (50ul) of the negative control, (Negative canine serum diluted in a stabilizing buffer) was added to wells C1 and D1. Then 50ul of each canine sera (canine serum samples), was added to the remaining wells. The microtiter plates was incubated for 90 mins ± 6 mins at 21^{oc} (±5^{oc}), after the incubation the well was emptied and each

washed 3 times with approximately 300ul of the wash solution (0.3M NaCL, 0.5M KCL, 0.13M Na2HPO4, 0.03M NaH2PO4, Tween 20 0.005%, PH 7.2). Conjugate 1× (anti-Pr-antibody peroxidase, horseradish peroxidase in PBS with 10% fetal bovine serum) was prepared by diluting the concentrated conjugate 10× to 1/10 in dilution buffer 2 and of the conjugate 1× which was added to each of the micro wells. The microtiter plate was then incubated for 30mins $\pm$ 3min at 21^{oc} (5^{oc}) after which the micro wells was emptied and each well was washed 3 times with approximately 300ul of the wash solution. Substrate solution, (Tetramethylbenzidine (TMB) and Oxygenated water in a sterilizing buffer), which is a revealing solution was added to each well. The microtiter plates were incubated again for 15 $\pm$ 2mins at $21^{o}C$ ($\pm5^{oc}$) in the dark and 100ul of stop solution (Sulphuric acid solution 0.5M) was added to each well in order to stop the reaction. The results were read and recorded using ELISA reader at an optical density of 450nm.

3.4.3.3 Reading and interpretation of ELISA results

Positive- In the presence of WNV antibodies, a colorless solution which remained colorless after incubation for 15minutes.

Negative-In the absence of antibodies, a blue solution which turned yellow after incubation for 15minutes appeared.

3.5 Data Analysis

Data collected from the study were analyzed using a statistical package for social sciences (SPSS version 2.0). Test of significance as chi-square and Fisher exact test were

used to detect the relationship between qualitative variables. Statistical significance was defined as a P-value of <0.05. Prevalence was calculated using the formula:

Prevalence=Number of individuals tested positive to microfilaria examination x 100

Total number of animals examined

Prevalence=Number of individuals tested positive to West Nile Virus antibodies x 100

Total number of animals examined

Results were analyzed using Chi-square (χ^2) and Fisher's exact test. A 95% confidence interval was set for the χ^2 values where appropriate to determine the association between the presence of the infection and age, sex, location, breed, and purpose.

Questionnaire data were analyzed using simple percentages to summarize data. Knowledge was assessed using the Likert method of questionnaire data analysis. Respondants who answered 4 questions correctly had good knowledge of questions asked, those who answered 3 questions correctly had fair knowledge those who answered 2 and below questions had poor knowledge of questions asked.

Comment [WU5]: What about the questionnaire data?
Secondly, how did you score or assessed knowledge

CHAPTER FOUR

4.0   **RESULTS**

A total of 360 dogs where sampled of which 30 samples were collected from each of the veterinary clinics; 45 samples from each of the two slaughter slabs in Kaduna metropolis and 90 samples from the slaughter slab in Zaria metropolis. Of the 180 dogs tested in the veterinary clinics. Local mongrel 336/360(93.3%) were the predominant breed of dogs sampled. Other breeds include 3/360(0.8%) bull dogs, 2/360(0.5%) bull mastiff; 2/360(0.5%) rottweiller; 7/360(1.9%) German shepherd; 5/360(1.3%) Italian mastiff; 2/360(0.5%) cross breed of mongrel and German shepherd; 1/360(0.2%) neopolitan mastiff; 1/360(0.2%) Russian shepherd and 1/360(0.2%) terrier (Table 4.1).

Table 4.1: Distribution of various breeds of dogs tested for Dirofilaria among dogs presented at Government-based Veterinary clinics and Slaughtered in Kaduna and Zaria metropolis

Breed	No sampled	Percentage % frequency
Mongrel	336	93.3
German shepherd	7	1.9
Bull mastiff	2	0.6
Neopolitan mastiff	1	0.3
Russian shepherd	2	0.6
Rottweiler	2	0.6
Italian mastiff	5	1.4
Terrier	1	0.3
Cross breed	2	0.6
Bull Dog	3	0.8
Total	**360**	**100**

4.1 Dirofilariasis

Four (1.1%) of the 360 blood samples analyzed were positive for dirofilarial parasite and these were observed among the mongrel breed and none in all the other breeds of dogs sampled (Table 4.1.1).

Table 4.1.1: Distribution of Dirofilaria in different breeds of dogs presented at Government-based Veterinary clinics and at Slaughtered in Kaduna and Zaria metropolis

Breed	No tested	No positive	% positive
Mongrel	336	4	1.1
German shepherd	6	0	0.0
Bull mastiff	2	0	0.0
Neopolitan mastiff	1	0	0.0
Russian shepherd	2	0	0.0
Rottweiller	2	0	0.0
Italian mastiff	5	0	0.0
Terrier	1	0	0.0
Cross breed	2	0	0.0
Bull dog	3	0	0.0
Total	**360**	**4**	**1.1**

df=10

Pvalue=1.000

X^2=0.2260

The dogs were classified into the following age groups <1 year, 1-2 years, 3-4 years, 5-6 years, >6 years. Dogs between the ages of 1-2 years showed a higher prevalence than older dogs. (Table 4.1.2).

Table 4.1.2: Distribution of Dirofilaria within age groups of dogs attending Government based Veterinary Clinics and at Slaughtered in Kaduna and Zaria Metropolis.

Age	No tested	No positive	Percentage positive
<1 year	50	1	2.0
1-2 years	208	3	1.4
3-4 years	68	0	0.0
5-6 years	23	0	0.0
>6 years	11	0	0.0
Total	**360**	**4**	**3.4**

Df=4

P=0.7936

X^2=1.684

The sex prevalence figures was 3(1.2%) for males and 1(0.8%) for females indicating a higher prevalence for males (Table 4.1.3).

Table 4.1.3: Distribution of Dirofilaria based on the sex of dogs attending Government based Veterinary Clinics and at Slaughtered in Kaduna and Zaria Metropolis.

Sex	No tested	No positive	Percentage positive
Male	241	3	1.2
Female	119	1	0.8
Total	**360**	**4**	**2.0**

OR=0.6751

RR=0.9960

Pvalue=1.000

Healthy looking dogs with body condition score of 2 and 3 had prevalence figures of 2(0.8%) and 2(1.9%) respectively (Table 4.1.4).

Table 4.1.4: Distribution of Dirofilaria based on the based on the body condition of dogs attending Government based Veterinary Clinics and at Slaughtered in Kaduna and Zaria Metropolis.

BCS	No tested	No positive	Percentage positive
1	12	0	0.0
2	243	2	0.8
3	101	2	1.9
Total	**360**	**4**	**2.7**

Df=2

X^2=0.9754

Pvalue=0.6140

Of the 180 dogs tested in the veterinary clinics, 2(6.7%) were positive from blood dirofilarial parasite in a clinic in Kaduna and 2(4.4%) from the slaughter slab located in Kaduna. (Tables 4.1.5.1; 4.1.5.2).

Table 4.1.5.1: Distribution of Dirofilaria in Dogs Attending Government Based
Veterinary Clinics in Kaduna and Zaria Metropolis

Clinic	No tested	No positive	Percentage positive
Abuth	30	0	0.0
Magajin Gari	30	0	0.0
Tudun wada Kaduna	30	2	6.7
Unguwan Rimi Kaduna	30	0	0.0
Sabo Kaduna	30	0	0.0
Zaria city	27	0	0.0
Total	**177**	**2**	**6.7**

Df=5

X^2=9.291

Pvalue=0.0980

Table 4.1.5.2: Distribution of Dirofilaria in Dogs Slaughtered In Kaduna and Zaria Metropolis

Slaughter	No tested	No positive	Percentage positive
Kaduna 1	45	2	4.4
Kaduna 2	44	0	0.0
Zaria	94	0	0.0
Total	**183**	**2**	**4.4**

Df=2

X^2=5.979

Pvalue=0.0503

The dogs tested were classified based on purpose into pets, hunting dogs and slaughter dogs; of which 2(1.0%) and 2(1.7%) dogs each used for slaughter and hunting respectively as at the period of this study were found positive with blood dirofilarial parasite (Table 4.1.6).

Table 4.1.6: Distribution of Dirofilaria among dogs attending government based veterinary clinics and at slaughter based on purpose in Kaduna and Zaria metropolis based on purpose

Purpose	No tested	No positive	Percentage positive
Slaughter	195	2	1.0
Hunting	120	2	1.7
Pet	45	0	0.0
Total	**360**	**4**	**2.7**

Df=2

X^2=0.8406

Pvalue=0.6669

4.2 West Nile Virus

A random sample of 88 samples were analyzed for the presence of West Nile virus anitibodies. Ten samples were collected from each of the veterinary clinics; eleven and ten samples from each of the two slaughter slabs in Kaduna metropolis and eleven from the slaughter slab in Zaria metropolis. The 4 positive samples for filarial was also included making a total of 92 samples. A total of 28 samples were found to be positive with West Nile virus antibodies and a single sample showed co-infection with both dirofilaria parasite and Westnile virus antibodies (Table 4.2.1).

Comment [WU6]: Define co-infection

Table 4.2.1: Seroprevalence of West Nile virus antibodies in Dirofilaria positive and randomly selected negative samples in dogs attending government based veterinary clinics and slaughters in Kaduna and Zaria

Filaria	**West Nile Virus Antibodies**		
	No tested	No positive	Percentage positive
Positive	4	1	25.0
Negative	88	27	30.7
Total	92	28	55.7

RR=1.045

OR=1.227

Pvalue=1.000

Comment [WU7]: Check font size and type

The seroprevalence showed a high percentage in dogs between the ages 1-2 years old for West Nile virus antibodies (Table 4.2.2). The difference in prevalence among the age groups was statistically significant (P<0.05).

Table 4.2.2: Seroprevalence of West Nile virus antibodies in Dirofilaria positive and randomly selected negative samples in dogs attending government based veterinary clinics and slaughters in Kaduna and Zaria based on age.

Age	No tested	No positive	Percentage positive
<1 years	23	1	4.3
1-2 years	23	18	78.3
3-4 years	23	7	30.4
>4 years	23	2	8.7
Total	92	28	121.7

Df=3

X^2=17.91

Pvalue=0.0005

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Ninety two serum samples were analyzed with an equal ratio of males and females. The seropositivity figures were 22(47.8%) for males and 6(13.0%) for females. The figure shows a high sex seroprevalence in males than females which is statistically significant (P<0.05) (Table 4.2.3).

Table 4.2.3: Seroprevalence of West Nile virus antibodies in Dirofilaria positive and randomly selected negative samples in dogs attending government based veterinary clinics and slaughters in Kaduna and Zaria based on sex.

Sex	No tested	No positive	Percentage positive
Male	46	22	47.8
Female	46	6	13.0
Total	92	28	60.8

RR=0.7647

OR=0.2727

Pvalue=0.0089

It was observed that seropositivity to West Nile virus antibodies occurred only in mongrel breed. The difference in prevalence was however not statistically significant (P>0.05) (Table 4.2.4).

Table 4.2.4: Seroprevalence of West Nile virus antibodies in Dirofilaria positive and randomly selected negative samples in dogs attending government based veterinary clinics and slaughters in Kaduna and Zaria based on breed.

Breed	No tested	No positive	Percentage positive
Mongrel	81	28	34.6
German shepherd	1	0	0.0
Bull mastiff	1	0	0.0
Neopolitan mastiff	1	0	0.0
Russian shepherd	1	0	0.0
Rottweiller	1	0	0.0
Italian mastiff	2	0	0.0
Terrier	1	0	0.0
Cross breed	1	0	0.0
Caucacian	1	0	0.0
Bull dog	1	0	0.0
Total	92	28	34.6

Df=9

X^2=3.359

Pvalue=0.9483

Of the 60 serum samples tested from the veterinary clinics, 10(16.7%) were seropositive for West Nile virus and 17(53.1%) of the 32 serum samples tested from the slaughter slabs. The difference in the prevalence based on location was not statistically significant P>0.05 and P>0.05 respectively (Table 4.2.5.1) and (Table 4.2.5.2 respectively).

Table 4.2.5.1: Seroprevalence of West Nile virus antibodies in Dirofilaria positive and randomly selected negative samples in dogs attending government based veterinary clinics

Clinic	No tested	No positive	Percentage positive
Abuth	10	3	30.0
Magajin gari	10	3	30.0
Tudun wada Kaduna	10	2	20.0
Sabo Kaduna	10	2	20.0
Unguwan rimi	10	0	0.0
Zaria	10	0	0.0
Total	**60**	**10**	**100**

Df=5

X^2=5.085

Pvalue=0.4055

Table 4.2.5.2: Seroprevalence of West Nile virus antibodies in Dirofilaria positive and randomly selected negative samples in dogs at slaughters in Kaduna and Zaria

Slaughter	No tested	No positive	Percentage positive
Kaduna 1	11	6	54.5
Kaduna 2	10	6	50.0
Zaria	11	6	54.5
Total	**32**	**18**	**159**

Df=2

X^2=0.02298

Pvalue=0.9886

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The dogs tested were classified based on purpose into pets, hunting dogs and slaughter dogs. 3(10.0%), 6(19.3%) and 19(61.2%) dogs as at the period of this study were found positive with West Nile virus antibodies (Table 4.2.6).

Table 4.2.6: Seroprevalence of West Nile virus antibodies among dogs attending government based veterinary clinics and at slaughter based on purpose in Kaduna and Zaria metropolis

Purpose	No tested	No positive	Percentage positive
Slaughter	31	19	61.2
Hunting	31	6	19.3
Pet	30	3	10.0
Total	**92**	**28**	**90.5**

Df=2

X^2=10.80

Pvalue=0.0045

Healthy looking dogs with body condition score of 2 and 3 had prevalence figures of 16(40.0%) and 8(20.0%) respectively (Table 4.2.7).

Table 4.2.7: Prevalence of West Nile virus in Dogs Attending Government Based Veterinary Clinics and Slaughtered and Kaduna and Zaria Metropolis Based On Body Condition Score

BCS	No tested	No positive	Percentage positive
1	12	4	33.3
2	40	16	40.0
3	40	8	20.0
Total	**360**	**28**	**93.3**

Df=2

X^2=2.076

Pvalue=0.3541

4.3 Questionnaire

Forty questionnaires were distributed to randomly selected dog owners to access their knowledge of non-malaria mosquito related diseases, attitudes and practice to dog care.

4.3.1 Demographic characteristics of respondants

One 1/40(2.5%) of the respondents was less than 20 years of age, 36/40(90.0%) were between the ages of 21-40 years old, 3/40(7.5%) were between 41-60 years old. Twenty seven 27/40(67.5%) were males and 13/40(32.5%) were females. Majority 35/40(87.5%) had attained up to tertiary level of education, 3(7.5%) had other forms of education and 2(5.0%) had no form of education. More than half 21/40(52.5%) of respondents kept local (mongrel) dogs, 8/40(20.0%) kept cross breeds, 11/40(27.5%) kept exotic breed of dogs. 18/40(45.0%) of the respondents were dog owners, 6/40(15.0%) were either spouses or children of the dog owners, 4/40(10.0%) were dedicated attendants of the dogs and 12/40(30.0%) had other relationships to the dog owners (Table 4.3.1).

Comment [WU8]: Rather too small. How did you calculate the questionnaire sample size?

Table 4.3.1: Demographic characteristics of questionnaire respondents sampled in Kaduna and Zaria metropolis

Variables	Frequency	Percentage (%)
AGE		
< 20 year	1	2.5
21 - 40 years	36	90.0
41 - 60 years	3	7.5
Total	**40**	**100.0**
SEX		
Male	27	67.5
Female	13	32.5
Total	**40**	**100**
EDUCATION		
Tertiary	35	87.5
Others	3	7.5
None	2	5.0
Total	**40**	**100**
BREED		
local	21	52.5
Cross	8	20.0
Exotic	11	27.5
Total	**40**	**100**
RELATIONSHIP WITH DOG		
dog owner	18	45.0
spouse or child of dog owner	6	15.0
dedicated dog attendant	4	10.0
Others	12	30.0
Total	**40**	**100**
DURATION OF RELATIONSHIP WITH DOG		
< 1 year	11	27.5
1 - 3 years	13	32.5
4 - 6 years	4	10.0
> 6 years	12	30.0
Total	**40**	**100**

4.3.2 Knowledge of non-malaria related diseases

Twelve 12/40(30.0%) of respondents agreed that diseases are transmitted from animals to humans via mosquito bites, 10/40(25.0%) disagreed, 18/40(45.0%) said they had no idea; 21/40(52.5%) agreed that are transmitted from humans to animals via mosquito bites, 6/40(15.0%) disagreed, 13/40(32.5%) said they had no idea; 20/40(50.0%) said mosquitoes cause diseases apart from malaria, 7/40(17.5%) said mosquitoes do not cause other diseases, 13/40(32.5%) said they had no idea; 24/40(60.0%) mosquitoes bite animals, 3/40(7.5%) said mosquitoes don't bite animals, 13/40(32.5%) said they had no idea (Table 4.3.2 and 4.3.3).

Table 4.3.2: Knowledge of respondents on mosquito borne diseases

Respondent	S	1	2	3	4	5	Total
1	M	20	0	0	0	0	20
2	M	0	0	0	0	0	0
3	M	0	0	20	0	0	20
4	M	20	0	20	20	0	60
5	F	0	0	0	20	0	20
6	F	0	0	0	0	0	0
7	F	0	0	0	0	0	0
8	M	20	0	0	0	0	20
9	M	20	10	20	20	0	70
10	M	0	0	0	0	0	0
11	M	0	0	20	20	0	40
12	M	0	0	20	20	20	60
13	M	20	20	20	20	20	100
14	M	20	20	20	20	20	100
15	M	0	0	0	0	0	0
16	F	20	0	20	20	20	80
17	M	20	20	20	20	0	80
18	M	0	0	20	0	0	20
19	F	20	20	20	20	20	100
20	M	20	20	20	20	20	100
21	M	20	20	20	20	20	100
22	M	20	20	20	20	20	100
23	F	0	0	0	0	0	0
24	M	20	10	20	0	0	50

25	F	20	10	20	20	0	70
26	M	0	0	20	20	0	40
27	M	0	0	0	0	0	0
28	M	0	0	20	0	0	20
29	M	0	0	0	0	0	0
30	M	0	0	0	0	0	0
31	M	0	0	0	0	0	0
32	F	0	0	0	0	0	0
33	F	0	0	20	20	20	60
34	F	20	0	20	20	20	80
35	F	20	20	20	20	20	100
36	M	20	20	20	20	20	100
37	F	20	0	20	20	20	80
38	F	20	20	20	20	20	100
39	F	20	0	20	0	0	40
40	M	0	0	0	0	0	0

$X^2 = 45.75$

Table 4.3.3: Knowledge of respondents on mosquito borne diseases

Variable	Frequency	Percentages
Are diseases transmitted from animals to human through mosquito bite?		
Yes	12	30.0
No	10	25.0
I don't know	18	45.0
Total	**40**	**100**
Are diseases transmitted from humans to animals through mosquito bite?		
Yes	21	52.5
No	6	15.0
I don't know	13	32.5
Total	**40**	**100**
Do mosquitoes cause other diseases apart from malaria?		
Yes	20	50.0
No	7	17.5
I don't know	13	32.5
Total	**40**	**100**
Do mosquitoes bite animals?		
Yes	24	60.0
No	3	7.5
I don't know	13	32.5
Total	**40**	**100**

4.3.4 Attitude and practice of respondents to dog care

Four 4/40(10.0%) of respondents reported that they always sighted ticks in or around their dog housing, 14/40(35%) reported that they frequently sighted ticks in or around their dog housing, 15/40(37.5%) rarely sighted ticks in or around their dog housing and 6/40(15.0%) never sighted ticks in or around their dog housing. Ten 10/40(25%) always sighted flies in or around their dog housing, 8/40(20%) frequently sighted flies in or around their dog housing, 13/40(32.5%) rarely sighted flies in or around their dog housing, 5/40(12.0%) never sighted flies in or around their dog housing. Seven 7/40(17.5%) always sighted mosquitoes in or around their dog housing, 6/40(15.0%) frequently sighted mosquitoes in or around their dog housing, 12/40(30.0%) rarely sighted mosquitoes in or around their dog housing, 11/40(27.5%) never sighted mosquitoes in or around their dog housing. Seven 7/40(17.5%) of respondents frequently sighted fleas in or around their dog housing, 18/40(45.0%) rarely sighted fleas in or around their dog housing, 9/40(22.5%) never sighted fleas in or around their dog housing (Table 4.3.4).

Four 4/40(10.0%) said that they and members of their families are always bitten by ticks, 9/40(22.5%) said they are rarely bitten by ticks and 24/40(60.0%) said they have never been bitten by ticks. Two 2/40(5.0%) said that they and members of their families are always bitten by flies, 4/40(10.0%) said they were frequently bitten by flies, 13/40(32.5%) said they were rarely bitten by flies and 17/40(42.5%) said they were never bitten by flies. Nine 9/40(22.5%) that they and members of their families are always bitten by mosquitoes, 16/40(40.0%) said they were frequently bitten by mosquitoes, 7/40(17.5%) said they were rarely bitten by mosquitoes and 5/40(12.5%) said they were

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never bitten by mosquitoes. Eight 8/40(20.0%) that they and members of their families are always bitten by rarely bitten by fleas and 26(65.0%) said they were never bitten by fleas (Table 4.3.5).

Table 4.3.4: Attitude and practice of respondents to dog care

VARIABLES	Frequency	Percentages (%)
How often do you see ticks in or around the dog housing?		
Always	4	10.0
Frequently	14	35.0
Rarely	15	37.5
Never	6	15.0
Total	**40**	**100**
How often do you see flies in or around the dog housing?		
Always	10	25.0
Frequently	8	20.0
Rarely	13	32.5
Never	5	12.0
Total	**40**	**100**
How often do you see mosquitoes in or around the dog housing?		
Always	7	17.5
Frequently	6	15.0
Rarely	12	30.0
Never	11	27.5
Total	**40**	**100**
How often do you see fleas in or around the dog housing?		
Always	0	0.0
Frequently	7	17.5
Rarely	18	45.0
Never	9	22.5
Total	**40**	**100**

Table 4.3.5: Practical knowledge of respondents to their health

Variables	Frequenc	Percentages
How often have you or members of your family been bitten by ticks?		
Always	4	10.0
Frequently	0	0.0
Rarely	9	22.5
Never	24	60.0
How often have you or members of your family been bitten by flies?		
Always	2	5.0
Frequently	4	10.0
Rarely	13	32.5
Never	17	42.5
Total	**36**	**90.0**
How often have you or members of your family been bitten by mosquitoes?		
Always	9	22.5
Frequently	16	40.0
Rarely	7	17.5
Never	5	12.5
Total	**37**	**92.5**
How often have you or members of your family been bitten by fleas?		
Always	0	0.0
Frequently	0	0.0
Rarely	8	20.0
Never	26	65.0
Total	**34**	**85.0**

Comment [WU9]: How did you score knowledge?

5.0   **DISCUSSION**

A prevalence rate of 1.1% dirofilarial infection is reported in dogs from this study. A

number of surveys for filarial parasites in dogs have been conducted in many countries in

the world with variations in prevalence rates. The findings in this study are different from

other reports from around the globe: 24.46% from Algiers, Algeria (Ben-Mahdi and

Mohamed, 2009), 12% from United States, 30% from Europe and between 46 to 59%

from Asia (Simon *et al*., 2012), 34% from Greece (Founta *et al*., 1999) and in Italy along

the Po river (more than 50%); higher than in the nearest Sardinia island (17%) (Rossi *et

al.,* 1996; Scala *et al*., 2005) and in Barcelona region in Spain (12.8%) (Aranda *et al*.,

1998), Tenerife islands (21%) (Montoya *et al.,* 2006) and was inferior to the one reported

in Las Palmas (36%) (Guerrero *et al*., 1989). Anyanwu *et al*. (2000) reported a

prevalence rate of 12.7% from Zaria, Nigeria, Chukwuebuka *et al*. (2016) 3.36% in

Nsukka, Nigeria, 2.15% in Markurdi, Nigeria (Ogbaje and Danjuma, 2016). The low

prevalence rate obtained in this study could be due to the difference in geographical

location, weather has been reported to be a critical factor in the prevalence of

dirofilariasis (Ogbaje and Danjuma, 2016). In addition, there is widespread and

indiscriminate use of antibiotics particularly the tetracycline antibiotics in Nigeria that

could reduce infection rate. The difference in prevalence observed for different parts of

Nigeria may also be due to the different types of tests used and skills of the

parasitologists. Transmission which depends on the intermediate host, a mosquito which

requires high relative humidity and an average temperature higher than 15°C, the *D.

immitis* larva, once ingested by mosquitoes, do not develop further unless at a regular

> **Comment [WU10]:** Dirofilaria infection or WNV infection?

temperature higher than 14°C (McCall *et al*., 2008). Kaduna metropolis and Zaria offers the ideal biotype to the mosquito vector proliferation and for the development of the larvae of *D. Immitis* (hot humid weather with annual temperature of 25.2 °C, 1211mm precipitation), stagnant water bodies and poor environmental sanitary conditions thus infection rates are expected to be higher than what was found in this study. However, the susceptibility of the local breeds that were predominant in this study may be different from breeds of dogs in other locations such as Europe that reported higher prevalences. Similarly, the dogs sampled are related to veterinary clinics, it is highly likely that the dogs have been subjected to routine deworming with antihelminthic agents in the dogs sampled. The Knotts technique is of low sensitivity compared to other detection methods (Chukwuebuka *et al*., 2016). There are also instances where infection could be present yet the dog is amicrofilaraemic. It is know that by the fact that dogs with prepatent or occult heartworm infections can be amicrofilaremic (Hoover *et al.,* 1996). The low prevalence of *D. immitis* in this study of 1.1% which was observed only in mongrel breed which is however lower than what was reported by Chukwuebuka *et al*. (2016) and also disagrees with his other findings that reported prevalence in cross breeds. However there is a correlation with the finding from this study and his which shows no sign of infection at all in exotic breeds of dogs studied. This may be due to the fact that owners of exotic breeds tend to give more proper attention to the administration of chemoprophylaxis, housing, purpose and general dwelling conditions than owners of local breeds.

It was observed that a higher prevalence of *D. immitis* was observed in hunting dogs followed by dogs at slaughter 1.7% and 1.0% respectively. This could be due to the fact that these group of dogs spend long hours outdoors and are left to scavenge for

themselves thereby exposing them to bites from insects eg mosquitoes thus agreeing with previous work carried out by Byeon *et al.* (2007).

According to (Brown *et al.*, 2012; Ahid *et al.,* 1999), high annual temperatures, suitable mosquito reproduction environment, and frequent migration of mosquitoes from other areas according to are some factors that contribute to a high rate of *D. immitis* infection (Brown *et al.*, 2012; Ahid *et al.,* 1999). Dogs are susceptible to various diseases, ailments, and poisons, some of which affect humans in the same way, others of which are unique to dogs. Dogs, like all mammals, are also susceptible to heat exhaustion when dealing with high levels of humidity and/or extreme temperatures (Gadahi *et al.,* 2008).

The bulk of this work was carried out during the dry season, with only one of the 4 positive cases observed in the dry season, this agrees with the findings of Greeve *et al.* (1983) who stated that dirofilariasis is usually associated with rainy season, largely due to preponderance of mosquitoes in the rainy season.

The higher incidence of the dirofilariasis in male dogs (1.2%) and older dogs (1.4%) agrees with previous report by CAPC (2015), who also reported higher rate of infection in male, older and exposed dogs, Selby *et al.* (1980) and Montoya et al. (1998) reported that male are more susceptible to infection by *D. immitis*. These are likely due to the exploratory life style of males, greater exposure time of adult dogs to insect bites and the long incubation period of the worm. Statistically, there were no significant differences

between sex and age groups in this study (P>1.00). This result is in agreement with the data provided by other authors (Oge *et al.*, 2003; Song *et al.*, 2003; Duran-Struuck *et al.*, 2005; Vieira *et al.*, 2014). The higher prevalence rate (4.04%) in outdoors system of dog management agrees with Guerrero (2012).

Evaluating the presence of *D. immitis* based on the general body condition score and general health of the dogs the high prevalence of positive cases in this study of (1.9%) in healthy dogs with a perfect body condition score of 3 followed by dogs with a normal body condition score of 2 (0.8%) agrees with Ben-Mahdi and Mohamed, (2009) who when evaluating the prevalence of *D. immitis* by general health status of the animals, obtained results which showed that only 24.44% of positive dogs were in bad general health, the others were in good general health and did not show any particular sign of disease. This suggests that a great majority of positive dogs (75.56%) were asymptomatic carriers. The proper pathological pattern of the canine dirofilariosis comes into play here due to the fact that the parasite takes up to 6 months to fully mature and the evolution of the disease could take years before any clinical manifestations appear. The slow and insidious evolution of this disease considerably lessens the quality of life and notably reduces the performances of these animals (McCall *et al.*, 2008). Thereby, clinicians should screen every animal that is not on preventive medication at the time of regular consultations. Because of the prognostic ineluctably dark of this affection, without any proper and effective medical treatment and the endemic evolution of this parasitosis within a canine population could have heavy medical and economical consequences particularly for all institutions using work dogs.

Little attention has been paid to dogs as hosts to West Nile virus hence the paucity of information on West Nile virus in dogs. However, considering the large populations of dogs and their close association with humans, understanding their clinical response to this infection and the likelihood that they might serve as amplifying hosts are important.

Previous studies carried out revealed that roughly one in three dogs from a West Nile Virus endemic region of South Africa were found to have neutralizing antibody to West Nile Virus (Blackwell *et al.,* 1989), 10 of 139 dogs sampled from New York City during the fall of 1999 were reported to have West Nile Virus neutralizing antibody (Komar *et al*., 1999); the number of these animals, that spent a considerable amount of timeout of doors was not known. This study however revealed that 28 out of the 92 dogs sampled were positive with the neutralizing antibody to West Nile virus which agrees with previous studies that dogs are capable of harboring the West Nile virus antibodies (Komar *et al*., 1999).

Aedesalbopictus mosquitoes were used to challenge dogs in an experiment because they are known to be capable of transmitting West Nile virus by bites and to reproduce a natural route of infection (Philip and Samdel, 1943; Akhter, 1982). In that study, none of the four dogs infected by mosquito bite showed clinical signs of disease, and although each became viremic, the quantity of virus in blood was low and fluctuated considerably. These results obtained by (Philip and Samdel, 1943; Akhter, 1982) are similar to those described by Blackwell and co-workers, who found no clinical signs and viremia in one of three dogs inoculated by subcutaneous and intravenous inoculation with a South African strain of West Nile virus (Blackwell *et al.,* 1989). Collectively, these observations and the paucity of clinical reports of West Nile virus disease in dogs suggest

that West Nile virus infection in dogs is typically subclinical in nature. This is in total agreement with this study because all of the dogs that tested positive to West Nile antibodies showed no clinical signs of illness.

The odds of seropositivity were approximately twice as great for stray dogs as for family dogs, and although not significant. Furthermore, outdoor-only family dogs and family dogs with a dog door had a higher West Nile virus seroprevalence than indoor-only family dogs. These findings indicate higher West Nile virus seroprevalence for animals with greater outdoor exposure (James *et al*., 2005). This totally agrees with the findings in this study were it was revealed that 13 slaughter dogs, 6 hunting dogs and 3 pet dogs were seropositive with West Nile virus antibodies.

The reason that the odds of seropositivity were 2.5 times as great in family dogs not receiving heartworm medication as those receiving heartworm medication could not be determined from the available information. Results suggest that environment-exposure variables do not explain this association. After adjusting for outdoor exposure, the overall association remained significant, indicating a consistent association between not receiving heartworm medication in family dogs and WNV seropositivity. Whether heartworm medication is protective against infection with WNV should be assessed.

Results of this study substantiate the need for further investigation of the potential use of dogs as sentinel indicators for WNV and the potential risk of human exposure. For arboviral diseases, a useful sentinel species for risk of human exposure would have similar vector-feeding patterns as humans, be highly susceptible to mosquito-borne infection yet resistant to disease, survive infection, develop detectable antibodies but not

develop sufficient viremia to infect mosquitoes, and not infect other species (Langevin *et al.,*2001;Apperson*et al.,* 2004). Domestic dogs fulfill these criteria (Austegen *et al.,* 2004; Blackburn, 1989).

> **Comment [WU11]:** Discuss the significance and importance of co-infection and increase transmissibility by co-infected mosquitoes.

A single case of co-infection with both *Dirofilaria* parasite and West Nile virus antibodies was recorded in this study and reason could be due to the problem of proximity to veterinary clinics, poor care and housing facility provided by the owner and a host of the other factors.

The questionnaire survey indicates a fair knowledge among the general public on mosquito borne illness and the vulnerability of animals to mosquito bites and mosquito borne illness and the transmission of illnesses from animals to humans and vice versa. This could be due to poor public health awareness. A better knowledge of mosquito borne illnesses was noticed among males than females. This could be due to the fact that the male child is given more attention to as regards education and also they spend more time with the dogs.

Due to similarities in the symptoms of these illnesses in human beings with malaria, they are often misdiagnosed by physicians resulting in wrong treatment and management practices thereby leading to mortalities or deformities which could have easily been avoidable.

A negligence of dog health among dog owners was also observed this could be due to the fact that a sense of value of dogs is lacking among dog owners here in Nigeria especially the mongrel breed.

> **Comment [WU12]:** Discuss the implication of lack of knowledge on other diseases transmitted by mosquitoes. The similarity of signs of these diseases with other common febrile illnesses such as malaria.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1. There is evidence of the presence of *Dirofilaria* spp in dogs attending government based veterinary clinics and slaughters in Kaduna metropolis and Zaria although at a low prevalence of 1.1%.

2. There is serological evidence of the presence of West Nile virus antibodies in dogs attending government based veterinary clinics and slaughters in Kaduna metropolis and Zaria.

3. There is evidence of co-infection of *Dirofilaria immitis* parasite and West Nile virus antibodies in dogs attending government based veterinary clinics and slaughters in Kaduna metropolis and Zaria indicating the ability of both infections to thrive in the same animal.

4. The higher prevalence (%) in mongrel breed of dogs compared to other exotic breeds indicates that the mongrel breed and subjected to inadequate domestic and medical care from the owners.

5. There was no significant association between age, sex, breed and filarial infection.

6. There was significant association between age and sex and West Nile virus infection.

7.   There is a poor awareness of non-malarial mosquito borne diseases which affect both humans and animals and also the ability of infections to be transmitted from humans to animals and vice versa by members of the public.

6.2 Recommendation

On the basis of the results obtained from this work, it is recommended that:

Dog owners and the general populace should be educated on the need to adhere to proper sanitary and hygienic conditions with their dogs.

Routine check up and use of anti parasitic drugs should be given to dogs especially the mongrel breed.

West Nile virus screening should be incorporated into routine screening of domestic animals.

Public health education on the different non malarial mosquito borne diseases and there modes of transmission especially via the human animal interface should be done.

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DEPARTMENT OF PUBLIC HEALTH AND PREVENTIVE MEDICINE

FACULTY OF VETERINARY MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA

CO-OCCURRENCE OF WNV AND FILARIA INFECTION IN HOUSEHOLD DOGS

Dear Respondent,

This questionnaire is designed as part of a scientific study of co-occurrence of WNV and Filaria infection in household dogs. The study intends to create an awareness of non-malarial diseases transmitted by mosquitoes to animals and humans alike.

If you accept to take part in the study, you will contribute in improving the public health awareness and understanding of diseases transmitted by mosquitoes and ways of prevention.

Kindly respond to the questions provided as accurately as possible. Your identity will not be required and all information obtained will be treated as confidential and used solely for academic purposes.

Thank you for your cooperation.

Fasanya Oluyinka O. A.

Department of Public Health and Preventive Medicine
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Zaria
07063103294

Please tick the appropriate option

1. Location
   a) Village/Ward……………………………………………………………………..
   b) Local Government
      Area……………………………………………………………………………
   c) Address…………………………………………………………………………..
   d) Tel No. of Respondent
      (Optional)……………………………………………………………………....

2. Age of the respondent
   a) Under 20 years
   b) 21-40 years
   c) 41-60 years
   d) Over 60 years

3. Sex of the respondent
   a) Male
   b) Female

4. Relationship with the dog
   a) Dog owner
   b) House help
   c) Gate keeper/maiguard

5. Level of training/education
   a) Primary school
   b) Secondary school
   c) Tertiary institution
   d) Islamiah
   e) None

6. How long have you been relating with the dog?
   a) Less than 3 months
   b) 3-6 months
   c) 6-9 months
   d) 9-12 months
   e) Over 12 months

7. What type of dog do you have?
   a) Local breed……………………………………………………………………....
   b) Foreign breed (please indicate species)……………………………………………

8. How do you house your dog?
   a) Inside your main building?
   b) Outside in a cage?

c) Left to roam about?

9. There are different types of mosquitoes
    a) Yes
    b) No
    c) I don't know

10. Mosquitoes transmit diseases via bites
    a) Yes
    b) No
    c) I don't know

11. Not all mosquitoes transmit diseases
    a) Yes
    b) No
    c) I don't know

12. Mosquitoes cause other diseases apart from malaria
    a) Yes
    b) No
    c) I don't know

13. Can you list some other disease apart from malaria caused by mosquitoes?
    a) ……………………………………………..
    b) ……………………………………………..
    c) ……………………………………………..

14. Animals are also bitten by mosquitoes
    a) Yes
    b) No
    c) I don't know

15. Diseases can be transmitted from animals to humans by mosquito bites
    a) Yes
    b) No
    c) I don't know

16. Diseases can be transmitted from humans to animals via mosquito bites
    a) Yes
    b) No
    c) I don't know